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THE THEORY OF MULTIPLE-STRAND CROSSING OVER[1]

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The Johns Hopkins University, Baltimore, Maryland

Received August 20, 1935

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THE PROBLEM

ACCORDING to the theory of the chiasmatype as originally formulated by JANSSENS (1909), interchange between homologous chromosomes takes place when each is already split longitudinally, but at any level only two of the four strands exchange parts. It was pointed out by MULLER (1916) and BRIDGES (1916) that the theory could be demonstrated genetically if it could be shown that in eggs which have retained two maternal strands, one strand may be a crossover and the other a non-crossover, or both crossovers but not at the same level. Such individuals were obtained by MULLER and BRIDGES; and although in these cases there was, as MULLER pointed out, the possibility that the extra strands had arisen by non-disjunction prior to maturation, the correctness of JANSSENS's theory has since been demonstrated by the regular occurrence of such individuals in races with attached X's or high non-disjunction and in triploids (ANDERSON 1925b, BRIDGES AND ANDERSON 1925, L. V. MORGAN 1925, REDFIELD 1930, STURTEVANT 1931).

If crossing over occurred at a two-strand stage, each chromatid would be identical with one of the other chromatids of the tetrad and the complement of the remaining two; hence the enumeration of the strands and the determination of how they are combined in the tetrad would be a simple matter. In four-strand crossing over, the strands recovered are presumably still a random sample of all the strands; but there are two complications. (1) Each of the missing strands is not necessarily either identical with or the complement of the strand recovered. (2) There is the possibility that crossing over may have occurred between sister strands; and this would not be directly detectable.

A complete theory of crossing over must take into account the missing strands in each tetrad and the unrecognizable crossings over. At first

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sight this may seem like a search for the substance of things hoped for, the evidence of things not seen; nevertheless the nature of the strands and tetrads can be deduced by calculation from the experimental data.

MATHEMATICAL METHOD

Definitions

Rank. The number of levels at which crossing over occurs in a strand or tetrad will be termed its rank. Non-crossovers are of rank 0, singles of rank 1, and so forth.

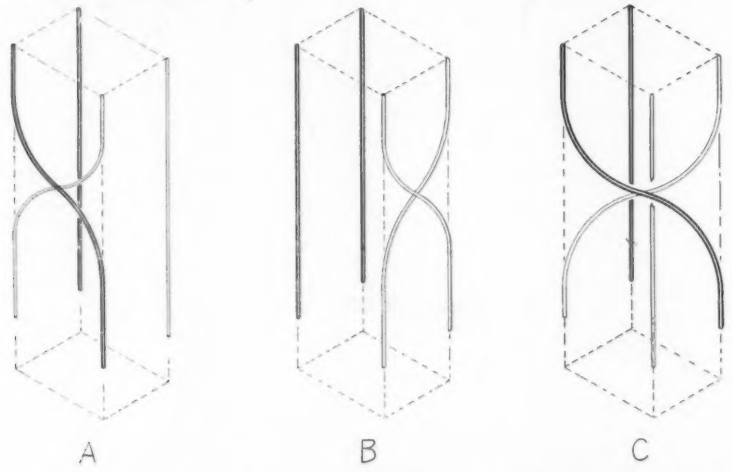


FIGURE 1. Types of single crossing over. A, lateral, involving homologous strands; B, lateral, involving sister strands; C, diagonal, involving homologous strands.

Types of single crossing over. A distinction must be made between homologous and sister-strand crossing over; also between lateral and diagonal crossing over if the strands of a tetrad are arranged along the edges of a quadrilateral prism (figure 1).

Types of multiple crossing over. In tetrads of rank 2, crossing over will be termed regressive, progressive, or digressive according to whether both or one or neither of the strands that cross over at the first level is involved in the crossing over at the second level (figure 2). A tetrad of higher rank than 2 may be mixed in type. In figure 3 are shown the various types of tetrads of ranks 2 and 3, but no distinction is made between lateral and diagonal crossing over.

A tetrad can give rise to strands of its own rank or of lower ranks but not to strands of higher rank. The rank of an emerging strand may however

be greater than the number of levels at which any one of the original strands that enter the tetrad crosses over (figure 3, tetrad G6).

Association of chromatids. Completely random association of chromatids in crossing over implies (1) that at any given level any two chromatids of a tetrad are equally likely to cross over (this may be termed random local association, or random occurrence of crossing over); (2) that the two chromatids which cross over at one level do not determine which shall cross over at other levels (random recurrence of crossing over).

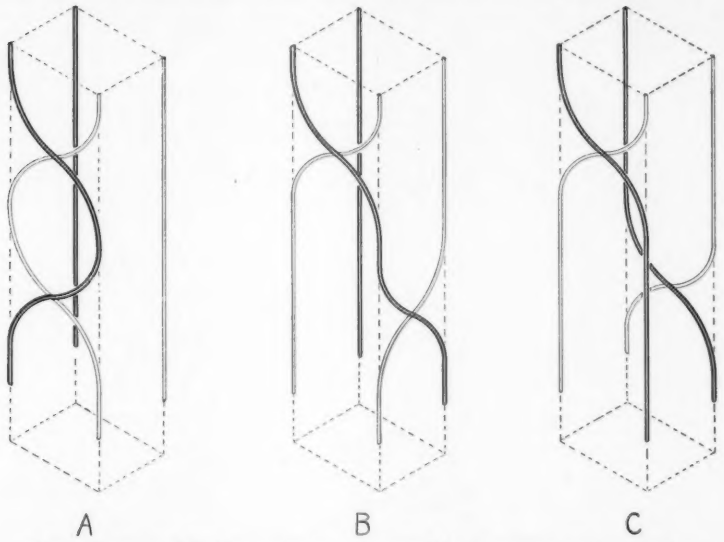


FIGURE 2. Types of double crossing over. A, regressive; B, progressive; C, digressive.

We shall first work out the theory of crossing over for completely random association; and then generalize it for cases where either occurrence, or recurrence, or both are not random.

Case 1. Recurrence random, chance of detecting crossing over constant.

A. Random occurrence (free sister-strand crossing over)

A tetrad of rank 0 can give rise only to non-crossover strands. In a tetrad of rank 1, half the strands will be crossovers and half non-crossovers, so that the chance of obtaining a strand of rank 1 will be 1/2, if elimination into polar bodies is a random matter. But not all the crossover strands will be recognizable as such: those resulting from crossing over between sister strands will remain unaltered. Since on a random basis one-third of the exchanges at a given level will be between sister

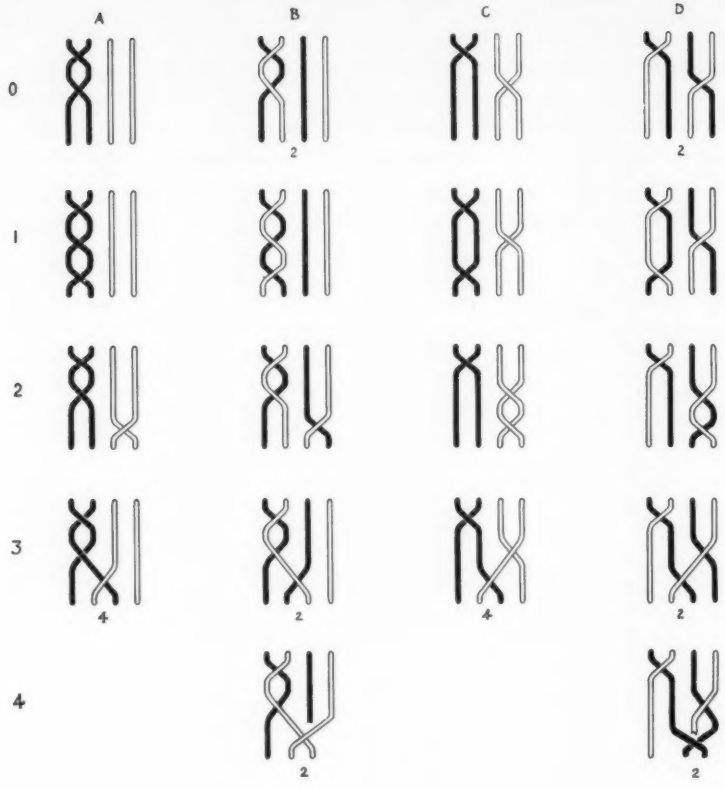
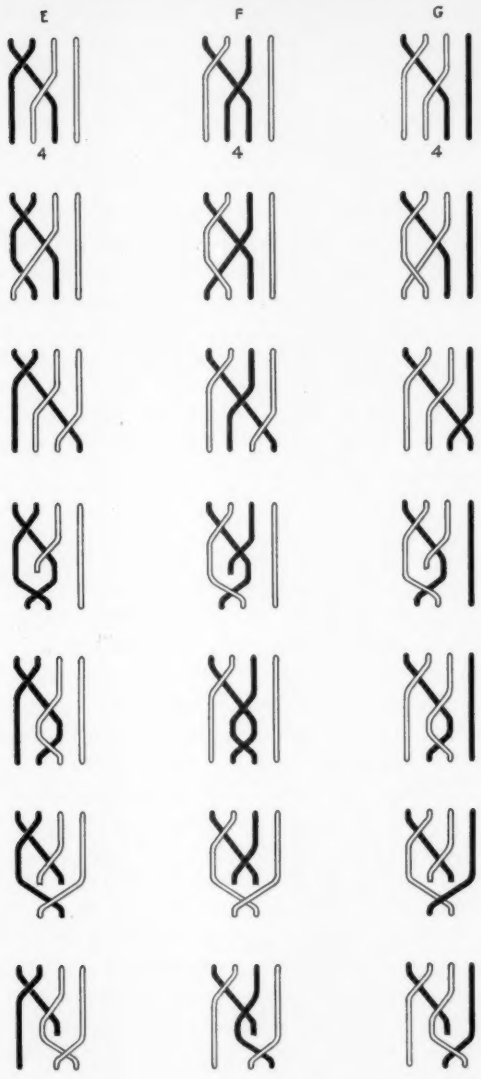


FIGURE 3. Types of double and triple crossing over. In the uppermost horizontal row (marked 0) are shown the types of tetrads of rank 2; the figure beneath each tetrad indicates its relative frequency. Beneath each tetrad of rank 2 are shown (rows 1–6) the tetrads that result when crossing over occurs in a third region. The numeral under each tetrad of rank 3 indicates its relative frequency among tetrads of rank 3 in the same vertical column; its frequency among all tetrads of rank 3 is the product of this number by the frequency of the tetrad of rank 2 from which it is derived. Where no number is indicated, 1 is understood. All frequencies in this figure are based on the assumption of random occurrence and recurrence.


E
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4
4

strands, only the other two-thirds will be recognizable. Hence the chance that a tetrad of rank 1 will give rise to a chromatid recognizable as a single crossover will be $1/2 \cdot 2/3 = 1/3$; that is, the observed frequency of crossing over in any region short enough to have only one crossing over at a time will be one-third of the true frequency.

If recurrence is random, the chances of detecting crossing over at different levels are independent. Hence the chance that a tetrad of rank 2 will result in an individual recognizable as a double crossover will be $1/3 \cdot 1/3 = 1/9$; the chance that a tetrad of rank 3 will result in an individual recognizable as a triple crossover will be $(1/3)^3 = 1/27$; and in general the chance that a tetrad of rank r will result in an individual recognizable as an r-ple crossover will be $(1/3)^r$.

The chance that a tetrad of rank 2 will result in an individual which is a recognizable crossover only in the first region is $1/3 \cdot 2/3 = 2/9$; and this is also the chance that the tetrad will give rise to an individual which is a recognizable crossover in the second region only. The chance that an individual will emerge which is a non-crossover or an apparent non-crossover will be $2/3 \cdot 2/3 = 4/9$.

Of strands derived from tetrads of rank 3, those that are recognizable as triple crossovers will be $(1/3)^3 = 1/27$; those that are recognizable as crossovers in the first two regions only will be $(1/3)^2\ 2/3 = 2/27$, and this will also be the frequency of strands that are recognizable crossovers in regions 1 and 3 only, or in 2 and 3 only. The frequency of recognizable crossovers in region 1 only will be $1/3\ (2/3)^2$; and this will also be the frequency of recognizable crossovers in region 2 only, or in region 3 only. Finally, the frequency of non-crossovers and apparent non-crossovers together will be $(2/3)^3 = 8/27$.

In general, if a tetrad is of rank r, the chance that it will give rise to a chromatid which is a recognizable crossover in some specified k of the r regions will be $(1/3)^k\ (2/3)^{r-k}$.

By this method we can deduce from a set of crossover data the frequencies of tetrads of different classes. In table 1, the experimental data in the first line are taken from a cross of BRIDGES (cited by WEINSTEIN 1918). Since each individual must have been derived from a tetrad of at least its own rank, the triples must be derived from tetrads of rank 3; for there were probably no tetrads of higher rank, because each of the three regions is too short to allow more than single crossing over within itself.

The one triple in the experimental data must represent 27 tetrads of rank 3. These tetrads must have produced in addition to the 1 triple, 2 individuals in each of the three rank-2 classes, 4 individuals in each class of rank 1, and 8 non-crossovers. If we subtract each of these from the total

in the corresponding class, the remainder represents those individuals in that class derived from tetrads of rank lower than 3.

We now turn to the 2, 3 class. The remainder in this class must be derived from tetrads that were crossovers in regions 2 and 3 only. From these tetrads emerged 41 individuals of class 2, 3; 82 that were crossovers in region 3 only, 82 that were crossovers in region 2 only, and 164 non-crossovers. In a similar way, we can calculate the individuals derived from the 1, 3 and the 1, 2 tetrads; and if we subtract all the individuals derived from tetrads of rank 2, the remainders represent the individuals derived from tetrads of lower ranks. The individuals in each crossover class of rank 1 constitute only 1/3 of those derived from the tetrads of the same class; the other 2/3 must have been non-crossovers and must be subtracted from the observed non-crossovers.

TABLE 1

Frequencies of tetrads calculated for random association including sister-strand crossing over.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	8	4	4	4	2	2	2	*1*
Remainders	9919	1945	1660	1647	86	205	41	
Frequencies and distribution of tetrads of rank 2	164		82	82			*41*	
	820	410		410		*205*		
	344	172	172		*86*			
Remainders	8591	1363	1406	1155				
Frequencies and distribution of tetrads of rank 1	2310			*1155*				
	2812		*1406*					
	2726	*1363*						
Remainder	*743*							
Corrected frequencies of tetrads	743	4039	4218	3465	774	1845	369	27

Thus we arrive at the italicized figures along the diagonal, which give the number of individuals of each class derived from tetrads of the same class.

These frequencies however are not the frequencies of the tetrads of each class; for, as we have seen, in a tetrad of rank r, only $(1/3)^r$ of the emerging strands are of the same rank, r. Hence it is necessary to multiply the remainder in each single crossover class by 3, in each double crossover class by 9, in each triple crossover class by 27. The results, in the lowest horizontal line, give the true number of tetrads in each class on the assumption of random occurrence and recurrence with sister-strand crossing over.

B. No sister-strand crossing over

If we assume that there is no crossing over between sister strands but that otherwise association of strands is random, crossing over may occur in one of four ways at any level (ab, ab′, a′b, a′b′, where a is the sister strand of a′ and b of b′). The chance that the strand recovered is a crossover is 1/2, and this is also the chance of detecting the crossing over at that level in the tetrad, since every crossing over is recognizable once it is obtained.

Of the strands derived from a tetrad of rank r, those that are also of rank r will be $(1/2)^r$, those that are crossovers in any $(r-1)$ specified regions will be $(1/2)^{r-1} \cdot 1/2$, and so forth. In general the chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of the r regions will be $(1/2)^k\ (1/2)^{r-k} = (1/2)^r$. That is, all the classes derived from tetrads of a given kind occur with equal frequency; since the frequency is independent of k, the number of regions in which the strands are crossovers.

This procedure is illustrated in table 2.

TABLE 2

Frequencies of tetrads calculated for random association without sister-strand crossing over.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	1	1	1	1	1	1	1	*1*
Remainders	9926	1948	1663	1650	87	206	42	
Frequencies and distribution of tetrads of rank 2	42		42	42			*42*	
	206	206		206		*206*		
	87	87	87		*87*			
Remainders	9591	1655	1534	1402				
Frequencies and distribution of tetrads of rank 1	1402			*1402*				
	1534		*1534*					
	1655	*1655*						
Remainder	*5000*							
Corrected frequencies of tetrads	5000	3310	3068	2804	348	824	168	8

The remainder in each class will now have to be multiplied by 2^r, where r is the rank of the class, to give the number of tetrads of that class.

C. The general case

It is conceivable that sister chromatids cross over with a frequency which differs from what it would be on a random basis but is not 0. The chance of detecting a crossing over in a region would then be neither 1/3

nor 1/2; let it be designated by p. Then if p has the same value in every region of the chromosome, the frequencies of tetrads can still be calculated by the method explained above.

A formula for this procedure can be derived as follows.

Let the chromosome be divided into regions so short that no crossing over of rank higher than 1 occurs within each. Let $a_0, a_1, a_2, \cdots a_n$ be the observed frequencies of individuals that are crossovers in 0, 1, 2, $\cdots$ n regions. It should be noted that each subscript represents the cardinal number of crossings over, not the ordinal number of the region in which crossing over takes place. Let $x_0, x_1, x_2, \cdots x_n$ represent the number of tetrads that are crossovers in 0, 1, 2, $\cdots$ n regions.

The chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of these r regions is $p^k (1-p)^{r-k}$, or $p^k q^{r-k}$ where $q = 1-p$. The number of ways in which these k regions can be specified is $[r(r-1)(r-2) \cdots (r-k+1)]/(1 \cdot 2 \cdot 3 \cdot \cdots k)$. Hence the total chance of obtaining a crossover strand of rank k from a tetrad of rank r is the product of these two expressions.

We can now form the following equations.

$$
\begin{aligned}
a_0 &= x_0 + qx_1 + q^2x_2 + q^3x_3 + q^4x_4 + q^5x_5 + \cdots + q^nx_n \\
a_1 &= px_1 + 2pqx_2 + 3pq^2x_3 + 4pq^3x_4 + 5pq^4x_5 + \cdots + npq^{n-1}x_n \\
a_2 &= p^2x_2 + 3p^2qx_3 + 6p^2q^2x_4 + 10p^2q^3x_5 + \cdots + C_2^n p^2 q^{n-2} x_n \\
a_3 &= p^3x_3 + 4p^3qx_4 + 10p^3q^2x_5 + \cdots + C_3^n p^3 q^{n-3} x_n \\
a_4 &= p^4x_4 + 5p^4qx_5 + \cdots + C_4^n p^4 q^{n-4} x_n \\
a_5 &= p^5x_5 + \cdots + C_5^n p^5 q^{n-5} x_n \\
&\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\
a_n &= p^n x_n
\end{aligned}
$$

In these $(n+1)$ equations there are $(n+1)$ unknowns, the x's; for the a's are observed frequencies and p is determined by the assumptions as to the chance of detecting a crossing over within a region. Hence if the equations are solved simultaneously, the x's can be evaluated.

But the value of x_0 can be obtained without solving for the other x's. For if the equations are multiplied respectively by $1, -q/p, q^2/p^2, -q^3/p^3, \cdots (-q/p)^n$, and then added together, the coefficients of every x except x_0 will add up to 0. Hence

$$x_0 = a_0 - \frac{q}{p} a_1 + \left(\frac{q}{p}\right)^2 a_2 - \left(\frac{q}{p}\right)^3 a_3 + \cdots + \left(-\frac{q}{p}\right)^n a_n. \qquad (1)$$

In this procedure a_0 has been used to represent the frequency of the non-crossovers. But it may be used for the frequency of any class; for ex-

ample, the crossovers in region 1, or the crossovers in regions 1 and 3, provided that the classes which are not crossovers in these regions are excluded. Then in the data so selected, $a_1, a_2, a_3, \cdots a_n$ represent the frequencies of the classes that are crossovers in the same region or regions as the a_0 class and in 1, 2, 3, $\cdots$ n additional regions; and $x_0, x_1, x_2, x_3, \cdots x_n$ are the frequencies of the tetrads which are crossovers in the same regions as the a_0 class and in 0, 1, 2, 3, $\cdots$ n additional regions.

The solution for x_0 will now give the number of tetrads of any specified class that gave rise to strands of the same class. But (unless this class is the non-crossovers) x_0 is not the total number of tetrads of the class; for there must have been others that gave rise to strands of lower rank and these have been excluded from the calculations. Since the proportion of tetrads of rank r that give rise to strands of the same rank is p^r, it is necessary to multiply x_0 by $1/p^r$ to get the number of all the tetrads of the class in question. This frequency is therefore

$$X=\frac{1}{p^r}x_0=\frac{1}{p^r}\left[a_0-\frac{q}{p}a_1+\left(\frac{q}{p}\right)^2a_2-\left(\frac{q}{p}\right)^3a_3+\cdots+\left(-\frac{q}{p}\right)^na_n\right]$$

$$=\frac{1}{p^r}\left[a_0-\left(\frac{1-p}{p}\right)a_1+\left(\frac{1-p}{p}\right)^2a_2\right.$$

$$\left.-\left(\frac{1-p}{p}\right)^3a_3+\cdots+\left(-\frac{1-p}{p}\right)^na_n\right]. \qquad (2)$$

where a_0 is the observed frequency of the class in question, and $a_1, a_2, a_3, \cdots a_n$ the observed frequencies of classes of additional rank 1, 2, 3, $\cdots$ n (WEINSTEIN 1928, 1932a).

For random occurrence and recurrence with sister strand crossing over, $p=1/3$ and the formula becomes

$$X=3^rx_0=3^r[a_0-2a_1+4a_2-8a_3+\cdots+(-2)^na_n]. \qquad (2a)$$

If sister-strand crossing over is entirely excluded but occurrence and recurrence are otherwise random $p=1/2$ and the formula becomes

$$X=2^rx_0=2^r[a_0-a_1+a_2-a_3+\cdots+(-1)^na_n]. \qquad (2b)$$

Case 2. Recurrence random, chance of detecting crossing over variable.

In case 1, it was assumed that p, the chance of detecting crossing over, is the same for all regions. It is theoretically possible however that the chance is not invariant, for sister chromatids might cross over in some regions and not in others, or more frequently in some regions than in others. Such differences might be caused by local conditions like proximity to the

end of the chromosome or to the spindle fibre or to inert regions or perhaps to particular genes.

In such cases we may designate the chance of detecting crossing over in regions 1, 2, 3, · · · n as p_1, p_2, p_3, · · · p_n. Let $q_1 = 1 - p_1$, $q_2 = 1 - p_2$, $q_3 = 1 - p_3$, · · · $q_n = 1 - p_n$.

If recurrence is random, the chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of the r regions is the product of the p's for the k regions and the q's for the remaining regions. Thus the chance that a tetrad which is a crossover in regions 1, 2, and 3 will give rise to a strand which is a crossover only in regions 1 and 3 is $p_1 p_3 q_2$.

The frequencies of tetrads can now be calculated as in table 3.

TABLE 3

Frequencies of tetrads calculated for random association except that sister strands cross over only in region 1.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	2	1	2	2	1	1	2	*1*
Remainders	9925	1948	1662	1649	87	206	41	
Frequencies and distribution of tetrads of rank 2	41		41	41			*41*	
	412	206		412		*206*		
	174	87	174		*87*			
Remainders	9298	1655	1447	1196				
Frequencies and distribution of tetrads of rank 1	1196			*1196*				
	1447		*1447*					
	3310	*1655*						
Remainder	*3345*							
Corrected frequencies of tetrads	3345	4965	2894	2392	522	1236	164	12

A formula for this procedure can also be deduced.

Let the observed frequency of the non-crossovers be represented by b_0; of the singles by b_1, b_2, b_3, · · · b_n; of the doubles by b_{12}, b_{13}, · · · b_{23}, b_{24}, · · · b_{34}, · · · $b_{(n-1)n}$; and similarly for the remaining classes. Each subscript now represents not, as before, the cardinal number of crossings over, but the ordinal numbers of the regions in which crossing over occurs. The frequencies of tetrads of various classes can be denoted by y with corresponding subscripts; thus y_0 is the frequency of non-crossover tetrads, y_1 of tetrads that are crossovers in region 1 only, and so forth.

We can now form a set of equations analogous to those in case 1. For

the sake of simplicity the equations will be given for only three regions; but the method is applicable to any number of regions.

$$
\begin{aligned}
b_0 &= y_0+q_1y_1+q_2y_2+q_3y_3+q_1q_2y_{12}+q_1q_3y_{13}+q_2q_3y_{23}+q_1q_2q_3y_{123}\\
b_1 &= p_1y_1 \qquad\qquad +p_1q_2y_{12}+p_1q_3y_{13} \qquad\quad +p_1q_2q_3y_{123}\\
b_2 &= \qquad p_2y_2 \qquad +p_2q_1y_{12} \qquad\quad +p_2q_3y_{23}+p_2q_1q_3y_{123}\\
b_3 &= \qquad\qquad p_3y_3 \qquad +p_3q_1y_{13}+p_3q_2y_{23}+p_3q_1q_2y_{123}\\
b_{12} &= \qquad\qquad\quad p_1p_2y_{12} \qquad\quad +p_1p_2q_3y_{123}\\
b_{13} &= \qquad\qquad\qquad\quad p_1p_3y_{13} \qquad +p_1p_3q_2y_{123}\\
b_{23} &= \qquad\qquad\qquad\qquad\quad p_2p_3y_{23}+p_2p_3q_1y_{123}\\
b_{123} &= \qquad\qquad\qquad\qquad\qquad\quad p_1p_2p_3y_{123}
\end{aligned}
$$

These equations can now be solved for the y's. But again it is possible to obtain the value of y_0 without solving for the other y's; for if the equations are multiplied respectively by

$$1, -\frac{q_1}{p_1}, -\frac{q_2}{p_2}, -\frac{q_3}{p_3}, \frac{q_1q_2}{p_1p_2}, \frac{q_1q_3}{p_1p_3}, \frac{q_2q_3}{p_2p_3}, -\frac{q_1q_2q_3}{p_1p_2p_3},$$

and then added together, the coefficients of the y's in every column, except y_0 will add up to 0. Hence

$$y_0=b_0-\left(\frac{q_1}{p_1}b_1+\frac{q_2}{p_2}b_2+\frac{q_3}{p_3}b_3\right)+\left(\frac{q_1q_2}{p_1p_2}\,b_{12}+\frac{q_1q_2}{p_1p_3}\,b_{13}+\frac{q_2q_3}{p_2p_3}\,b_{23}\right)-\frac{q_1q_2q_3}{p_1p_2p_3}\,b_{123}. \tag{3}$$

If the b_0 class is of rank 0, then y_0 is the number of non-crossover tetrads. But as in case 1, the method can be applied to part of the data: then b_0 is the frequency of a crossover class and y_0 is the number of tetrads of this class that gave rise to chromatids of the same class. Hence y_0 must be divided by the product of the p's for the regions in which the class is a crossover. These regions are not any of those numbered from 1 to 3, for the b_0 or y_0 class is not a crossover in 1 or 2 or 3. Hence the regions in which the class is a crossover can be numbered separately from 1 to r, where r is the rank of the class. The chance of detecting crossing over in each of these r regions may be denoted by p' with the proper subscript; and the total number of tetrads of the class now becomes

$$X=\frac{1}{p_1'p_2'p_3'\cdots p_r'}\,y_0. \tag{4}$$

If the equations had included n regions, the general formula for the number of tetrads of any class would be

$$X=\frac{1}{p_1'p_2'p_3'\cdots p_r'}\left[b_0-\left(\frac{q_1}{p_1}\,b_1+\frac{q_2}{p_2}\,b_2+\cdots+\frac{q_n}{p_n}\,b_n\right)\right.$$
$$+\left(\frac{q_1q_2}{p_1p_2}\,b_{12}+\frac{q_1q_3}{p_1p_3}\,b_{13}+\cdots+\frac{q_2q_3}{p_2p_3}\,b_{23}+\cdots+\frac{q_{(n-1)}q_n}{p_{(n-1)}p_n}\,b_{(n-1)n}\right)$$
$$\left.+\cdots+(-1)^n\,\frac{q_1q_2q_3\cdots q_n}{p_1p_2p_3\cdots p_n}\,b_{123\cdots n}\right]. \qquad (5)$$

Case 3. Recurrence not random, chance of detecting crossing over constant.

The frequencies of regressive, progressive, and digressive crossing over are not necessarily determined by chance alone: it is conceivable that they may vary with the nature of the crossings over (whether homologous or sister strand, lateral or diagonal), with their distance apart, and with the particular regions involved. They might also depend on other crossings over: their number, their distance away, the regions in which they occur, and their nature (including now not only whether they are homologous or sister-strand, and lateral or diagonal, but also whether they are regressive, progressive, or digressive with respect to the crossings over under consideration).

In making a table like tables 1 and 2, we may therefore subdivide each class of tetrad into its different types (such as are shown in figure 3) and distribute separately the chromatids emerging from each type.

If when we are considering tetrads of a given class we add together all the emerging strands that are crossovers in some specified k of the r regions, the proportion of such strands is no longer $p^r\,q^{r-k}$. The ratio of the actual proportion to the proportion expected on random recurrence may be designated by t; its value will in the most general case vary with the regions of crossing over in the tetrad and in the emerging chromatid; these regions may therefore be indicated by numerical subscripts, positive if the crossing over of the tetrad appears in the chromatid, negative if it does not. For example, the chance that a tetrad which is a crossover in regions 1, 2, and 3 will give rise to a chromatid which is a crossover in regions 1 and 3 only may be written as $t_{1-2+3}\,p^2\,q$.

In a table like table 1 the proper value of t will enter into the frequency of each class if recurrence is not random; for example, the distribution of tetrads of rank 3 will be as follows:

Regions of crossing over	0	1	2	3	12	13	23	123
Frequencies and distribution of tetrads of rank 3	$8t_{-1-2-3}$	$4t_{1-2-3}$	$4t_{-1+2-3}$	$4t_{-1-2+3}$	$2t_{1+2-3}$	$2t_{1-2+3}$	$2t_{-1+2+3}$	t_{1+2+3}

The x_0 values (the figures on the diagonal) for classes of rank 2 or more must be divided not merely by p^r but by the product of p^r and the ap-

propriate t: the remainder in the 123 column must be divided by $t_{1+2+3}p^3$, that in the 23 column by $t_{2+3}p^2$, and so on.

The t's will also enter into the equations corresponding to those on page 163; this will be considered in connection with the next case. The general formula derived from the equations will now be more complicated; but if ranks above 2 are absent, the frequency of tetrads of rank 0 is

$$y_0 = a_0 - \frac{q}{p} a_1 + \frac{t_{1-2} + t_{-1+2} - t_{-1-2}}{t_{1+2}} \frac{q^2}{p^2} a_2. \tag{6}$$

Case 4. Recurrence not random, chance of detecting crossing over variable.

The same considerations apply to this case as to case 3 and the t factors enter into it in the same way. Thus the chance that a tetrad of rank 3 will give rise to a strand which is a recognizable crossover in regions 1 and 3 only will be $t_{1-2+3}p_1p_3q_2$.

With non-random recurrence, the distribution of tetrads of rank 3 in table 3 would be as follows:

Regions of crossing over	0	1	2	3	12	13	23	123
Frequencies and distribution of tetrads of rank 3	$2t_{-1-2-3}$	t_{1-2-3}	$2t_{-1+2-3}$	$2t_{-1-2+3}$	t_{1+2-3}	t_{1-2+3}	$2t_{-1+2+3}$	t_{1+2+3}

and each x_0 value must be divided by the product of $p_1'p_2' \cdots p_r'$ and the appropriate t.

The equations on page 166 will now become as follows.

$$\begin{aligned}
b_0 &= y_0 + q_1y_1 + q_2y_2 + q_3y_3 + t_{-1-2}q_1q_2y_{12} + t_{-1-3}q_1q_3y_{13} + t_{-2-3}q_2q_3y_{23} + t_{-1-2-3}q_1q_2q_3y_{123} \\
b_1 &= p_1y_1 + t_{1-2}p_1q_2y_{12} + t_{1-3}p_1q_3y_{13} + t_{1-2-3}p_1q_2q_3y_{123} \\
b_2 &= p_2y_2 + t_{-1+2}p_2q_1y_{12} + t_{2-3}p_2q_3y_{23} + t_{-1+2-3}p_2q_1q_3y_{123} \\
b_3 &= p_3y_3 + t_{-1+3}p_3q_1y_{13} + t_{-2+3}p_2q_2y_{23} + t_{-1-2+3}p_3q_1q_2y_{123} \\
b_{12} &= t_{1+2}p_1p_2y_{12} + t_{1+2-3}p_1p_2q_3y_{123} \\
b_{13} &= t_{1+3}p_1p_3y_{13} + t_{1-2+3}p_1p_3q_2y_{123} \\
b_{23} &= t_{2+3}p_2p_3y_{23} + t_{-1+2+3}p_2p_3q_1y_{123} \\
b_{123} &= t_{1+2+3}p_1p_2p_3y_{123}
\end{aligned}$$

The formula for y_0 is now still more complicated; but if crossovers of ranks above 2 are absent or neglected,

$$\begin{aligned}
y_0 = b_0 - \left(\frac{q_1}{p_1}b_1 + \frac{q_2}{p_2}b_2 + \cdots + \frac{q_n}{p_n}b_n\right) \\
+ \frac{t_{1-2} + t_{-1+2} - t_{-1-2}}{t_{1+2}} \frac{q_1q_2}{p_1p_2} b_{12} + \frac{t_{1-3} + t_{-1+3} - t_{-1-3}}{t_{1+3}} \frac{q_1q_3}{p_1p_3} b_{13} \\
+ \cdots + \frac{t_{(n-1)-n} + t_{-(n-1)+n} - t_{-(n-1)-n}}{t_{(n-1)+n}} \frac{q_{(n-1)}q_n}{p_{(n-1)}p_n} b_{(n-1)n}.
\end{aligned} \tag{7}$$

In this formula, as in the equations from which it is derived, y_0 and b_0

represent respectively the number of tetrads and of chromatids of rank 0. If b_0 is used for a class of higher rank, the equations must be modified by omitting every term in which the y is not a crossover in at least the same regions as the b_0 class. The y's can then be renumbered so that the class of lowest rank is y_0, and a general formula can be derived as in cases 1 and 2. This, however, is complicated; and the frequencies of tetrads of classes ranking above 0 can be obtained by solving for the y's in the equations as they stand.

The frequency of chromatids

The observed frequency of any class includes of course only the chromatids that are homologous-strand crossovers in the specified regions. The true frequency of chromatids of the class would include sister-strand crossovers as well.

The relations between tetrad frequencies and true frequencies of chromatids are given by the sets of equations on pages 163, 166, and 168, and by equations 1–7 if the x's and y's retain their original meanings, but in the definitions of the a's, b's, p's, and t's the true frequencies of chromatids are substituted for the observed frequencies. The value of p will now vary from 1/2 when at any level of crossing over only two strands are involved to 1 when four strands are always involved in pairs.

The sets of equations on pages 163, 166, and 168, and equations 1–7 also express the relations between the observed and the true frequencies of chromatids if the a's and b's denote observed frequencies, the x's and y's the true frequencies of chromatids (not of tetrads), if each p denotes what proportion of exchanges are between homologous strands, and if the t's are modified accordingly (WEINSTEIN 1928, 1932a).

Each p as originally defined is of course the product of the p's of the two preceding paragraphs.

ORDINARY DIPLOIDS

The results of applying the multiple-strand method to ordinary diploids are illustrated in tables 4 and 8.

Table 4 is based on a cross involving almost the entire length of the X chromosome of *Drosophila melanogaster* (*sc ec cv ct v g f*). The experimental data (column A) comprise 28239 individuals, including 24034 from BRIDGES and OLBRYCHT (1926), 2047 from ANDERSON (1925a, table IV), and 2158 from an experiment (hitherto unpublished) by the writer. In the other columns are given tetrad frequencies calculated on the assumption of random recurrence for various values of p. The column headed $p = 1/2$ is based on the assumption that sister chromatids do not cross over (formula 2b); that headed $p = 1/3$ on the assumption that they cross over as freely

TABLE 4

Crossing over involving sc ec cv ct v g f (28239 individuals).

REGIONS OF CROSSING OVER	OBSERVED CHROMATID FREQUENCIES* p=2/3	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE							
		p=2/3	p=1/2	p=1/3 IN REGIONS INDICATED, 1/2 IN OTHER REGIONS					p=1/3
				1	12	123	1234	12345	
	A	B†	C	D	E	F	G	H	I
0	12776	6716	1709	837	−459	−2007	−4392	−5305	−5316
1	1407	1696	1744	2616	2601	2577	2220	1707	1203
2	2018	2483	2602	2592	3888	3864	3492	2724	1836
3	1976	2649	3130	3112	3096	4644	4527	4116	3546
4	3378	4515	5328	5094	4848	4770	7155	6861	6102
5	2356	2951	3180	2812	2296	2022	1826	2739	2610
6	2067	2284	1998	1626	1016	616	108	22	33
12	9	15	20	30	45	36	45	45	45
13	16	27	36	54	48	72	90	63	54
14	142	291	468	702	702	714	1071	981	846
15	198	430	736	1104	1104	1086	1026	1539	1530
16	206	440	744	1116	1110	1104	1014	1008	1512
23	11	21	36	32	48	72	81	81	99
24	136	291	492	492	738	744	1116	1098	1008
25	261	584	1032	1032	1548	1548	1536	2304	2295
26	318	701	1224	1220	1830	1842	1782	1776	2664
34	42	88	148	152	156	234	351	351	306
35	148	324	560	548	548	822	822	1233	1188
36	212	463	800	792	800	1200	1170	1140	1710
45	123	262	440	400	392	392	588	882	873
46	315	674	1136	1076	1036	1016	1524	1518	2277
56	59	124	204	200	196	176	172	258	387
123	3	7	8	12	18	27			−27
124	1	2				−18	−27	−27	−27
126	2	5	8	12	18				
134		−2	−8	−12	−24	−36	−54	−54	−54
135	3	10	24	36	36	54	54	81	81
136	3	8	16	24	12	18	18	18	27
145	10	34	80	120	120	120	180	270	270
146	15	51	120	180	180	180	270	270	405
156	1	3	8	12	12	12	12	18	27
234	1	2		−8	−12	−18	−27	−27	−27
236		−2	−8	−16	−24	−36	−36	−36	−54
245	2	7	16	16	24	24	36	54	54
246	10	34	80	80	120	120	180	180	270
256	1	3	8	8	12	12	12	18	27
346	5	17	40	40	40	60	90	90	135
356	5	17	40	40	40	60	60	90	135
456	1	3	8	8	8	8	12	18	27
1234	1	5	16	24	36	54	81	81	81
1236	1	5	16	24	36	54	54	54	81

† In the p=2/3 column, the only one in which fractional values occur, they are given to the nearest unit. The values in the p=2/3 column also represent the true frequencies of chromatids when p=1/3.

* From BRIDGES and OLBRYCHT 1926, ANDERSON 1925a, and WEINSTEIN.

as other chromatids (formula 2a). In the latter column the frequency of the non-crossover class is negative; and since this is impossible, it follows that there cannot be random association of chromatids with free crossing over between sister strands.

The results are of course subject to errors of sampling as well as errors due to differential viability and other causes. These errors may become exaggerated in the course of the calculations because the observed frequencies of the crossovers of higher rank are small and are multiplied by relatively large factors, so that slight differences may be magnified. For this reason the negative frequencies among the crossovers (they are all among the triples) are obviously not significant, for they would become 0 or positive with small changes in the observed numbers of triples or quadruples, or in some cases if the quadruples were derived from tetrads of rank 5.

The errors of sampling may be calculated by the formula for the standard error of a function of several variables, which may be written

$$E=\sqrt{\left(\frac{\partial F}{\partial v_0}\right)^2 e_0^2+\left(\frac{\partial F}{\partial v_1}\right)^2 e_1^2+\left(\frac{\partial F}{\partial v_2}\right)^2 e_2^2+\cdots+\left(\frac{\partial F}{\partial v_n}\right)^2 e_n^2}$$

where F is a function of the v's, E its standard error, and the e's are the standard errors of the respective v's. This formula holds for all cases where the distribution of errors is Gaussian (SCARBOROUGH 1930, pp. 337–338). For F we may substitute the tetrad frequency X; and for the v's we may substitute the a's of formula 2 or 2a or 2b, or the b's of formula 5.

The frequency of the non-crossovers in the $p=1/3$ column is approximately 20 times its standard error, which is 268. The result cannot therefore be due to errors of sampling. Nor can it be due to differential viability, for viability was good in the experiments on which the calculations are based.

We may conclude that sister strands do not cross over as freely as homologous strands if the association of chromatids in crossing over is otherwise random. It does not follow however that they do not cross over at all: they might cross over only in some regions, or throughout the chromosome but to a smaller extent than homologous strands.

To test the first of these alternatives, tetrad frequencies were calculated by means of formula 5 for $p=1/3$ (free sister-strand crossing over) in some regions and $p=1/2$ (no sister-strand crossing over) in others. The results are given in columns D–H of table 4. As sister-strand crossing over is restricted to a shorter and shorter region at the left of the X chromosome, the negative frequencies approach 0 and finally become positive. The negative frequencies are from about 10 to about 20 times their standard errors, except −459, which is about 2.5 times its standard error. Thus it is shown

that free crossing over between sister chromatids, if it occurs at all, must be limited to a short region. This is not necessarily at the left end of the X, for the results are similar if we postulate sister-strand crossing over in other regions.

There remains the possibility that recurrence is not random. This can be tested by seeing whether the relative frequencies of tetrads can be altered without changing the frequencies of strands as given by experiment.

TABLE 5

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them, on the assumption of random association without sister-strand crossing over ($p=1/2$).

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	1709	1709		
1	17982	1/2=8991	1/2=8991	
2				
1/4=regressives	2019	1/2=1009.5		1/2=1009.5
1/2=progressives	4038	1/4=1009.5	1/2=2019	1/4=1009.5
1/4=digressives	2019		2019	

Table 5 gives the frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of random association without sister-strand crossing over. The following equations indicate what combinations of tetrads are equivalent with respect to the strands derived from them:

$$1 \text{ non-crossover} + 1 \text{ digressive} = 2 \text{ singles.} \tag{8}$$

$$1 \text{ regressive} + 1 \text{ digressive} = 2 \text{ progressives.} \tag{9}$$

$$1 \text{ non-crossover} + 2 \text{ progressives} = 1 \text{ regressive} + 2 \text{ singles.} \tag{10}$$

Still other substitutions are possible if ranks above 2 are included; for example,

$$1 \text{ single} + 1 \text{ regressive} = 1 \text{ non-crossover} + 1 \text{ digressive (or regressive-digressive) of rank 3.} \tag{11}$$

By a rank-3 digressive is meant a tetrad like D1 in figure 3; by a rank-3 regressive-digressive is meant one like D2 or B2.

In making these substitutions, it is necessary to consider the regions of crossing over, so that the proper classes and frequencies of strands may result; also in order to avoid digressives with two crossings over in the same region, unless it is desired to test the possibility of such digressives. The substitutions are also limited by the frequencies of tetrads to be replaced; hence it follows that deviations from random recurrence will, if too great, lead to negative frequencies of tetrads. Nevertheless considerable deviations are possible without changes in the frequencies of strands;

but coincidence of tetrads will be altered, as will also the frequencies of progeny of attached X's. These results will be considered below.

Equations 8 and 11 hold when sister strands cross over freely; the other equations now assume the following forms:

$$1 \text{ regressive} + 3 \text{ digressives} = 4 \text{ progressives.} \quad (12)$$

$$3 \text{ non-crossovers} + 4 \text{ progressives} = 1 \text{ regressive} + 6 \text{ singles} \quad (13)$$

If sister strands cross over freely, then as can be seen from table 6, random recurrence results in an excess of tetrads of ranks higher than 0 and of non-crossover strands derived from them, so that a negative frequency of tetrads of rank 0 must be postulated to bring the total number of tetrads and of non-crossover strands down to the observed figure. The negative frequency will disappear if non-random recurrence can reduce by 5316 the number of those tetrads of ranks higher than 0 that give rise to non-crossover strands. The simplest way to do this is to replace 10632 tetrads of rank 1 by their equivalent 5316 non-crossover tetrads and 5316 digressives, in accordance with equation 8.

TABLE 6

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of random association including sister-strand crossing over ($p = 1/3$).

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	−5316	−5316		
1	15330	2/3 = 10220	1/3 = 5110	
2				
1/6 = regressive	2799	2/3 = 1866		1/3 = 933
2/3 = progressive	11196	5/12 = 4665	1/2 = 5598	1/12 = 933
1/6 = digressive	2799	1/3 = 933	2/3 = 1866	

TABLE 7

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of non-random association with sister strand crossing over ($p = 1/3$). (For explanation see text.)

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	0	0		
1	4698	3132	1566	
2				
regressive	2799	1866		933
progressive	11196	4665	5598	933
digressive	8115	2705	5410	

The tetrad and strand frequencies as revised by this method are given in Table 7. They may be modified by other substitutions.

TABLE 8

Crossing over involving sc ec cv ct v s f car bb (16136 individuals).

REGIONS OF CROSSING OVER	OBSERVED CHROMATIN FREQUENCIES*	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE									
				p=1/3 in regions indicated, 1/2 in other regions							p=1/3
		p=2/3	p=1/2	1	12	123	1234	12345	123456	1234567	
	A	B†	C	D	E	F	G	H	I	J	K
0	6607	3300	904	697	175	−301	−1161	−1547	−1683	−1462	−1339
1	506	530	414	621	618	609	501	357	18	−120	−204
2	1049	1167	1046	1044	1566	1566	1497	1266	624	327	189
3	855	996	960	952	952	1428	1371	1230	717	483	399
4	1499	1817	1876	1804	1758	1720	2580	2466	1914	1494	1305
5	937	1143	1196	1096	942	848	772	1158	1080	981	903
6	1647	1867	1710	1480	1036	692	324	272	408	363	333
7	683	661	420	320	100	−66	−346	−412	−442	−663	−651
8	379	350	202	140	24	−42	−180	−234	−254	−246	−369
12	3	4	4	6	9	0	0	0	−9	−9	−9
13	6	11	16	24	18	27	27	18	18	18	18
14	41	87	144	216	216	216	324	315	306	288	279
15	55	118	200	300	300	294	288	432	432	414	405
16	118	262	460	690	684	684	678	678	1017	1017	1008
17	54	117	200	300	300	300	288	276	276	414	414
18	34	73	124	186	186	186	180	174	168	168	252
23	3	4	4	0	0	0	0	0	−9	−9	−9
24	38	69	92	92	138	138	207	207	171	126	72
25	85	182	308	308	462	462	462	693	666	630	621
26	237	517	892	888	1332	1326	1302	1284	1926	1908	1881
27	123	262	440	440	660	660	630	606	594	891	873
28	70	144	232	232	348	348	312	306	288	276	414
34	22	46	76	76	76	114	171	171	171	153	144
35	55	116	192	188	188	282	282	423	414	396	369
36	177	394	692	692	688	1032	1032	1026	1539	1530	1521
37	88	192	332	332	332	498	486	474	468	702	702
38	38	80	132	132	132	198	192	174	168	168	252
45	41	90	156	152	152	152	228	342	342	342	333
46	198	435	756	752	736	736	1104	1104	1656	1656	1620
47	159	346	596	588	568	560	840	840	840	1260	1251
48	91	189	308	304	280	276	414	408	384	378	567
56	35	73	120	120	108	104	104	156	234	234	225
57	49	101	164	156	140	132	132	198	198	297	297
58	40	82	132	128	124	112	108	162	156	156	234
67	21	44	72	72	64	60	60	60	90	135	135
68	30	56	80	76	64	60	44	40	60	60	90
78	2	1	−4	−4	−12	−12	−16	−16	−16	−24	−36

* From BRIDGES. † See footnote to table 4.

TABLE 8 (*Continued*)

Crossing over involving sc ec cv ct v s f car bb (16136 individuals).

	A	B	C	D	E	F	G	H	I	J	K
123	1	3	8	12	18	27	27	27	27	27	27
126	1	3	8	12	18	18	18	18	27	27	27
135	1	3	8	12	12	18	18	27	27	27	27
145	1	3	8	12	12	12	18	27	27	27	27
146	1	3	8	12	12	12	18	18	27	27	27
147	2	7	16	24	24	24	36	36	36	54	54
148	1	3	8	12	12	12	18	18	18	18	27
157	2	7	16	24	24	24	24	36	36	54	54
158	1	3	8	12	12	12	12	18	18	18	27
168	1	3	8	12	12	12	12	12	18	18	27
236	1	3	8	8	12	18	18	18	27	27	27
246	4	13	32	32	48	48	72	72	108	108	108
247	5	17	40	40	60	60	90	90	90	135	135
248	6	20	48	48	72	72	108	108	108	108	162
256	3	10	24	24	36	36	36	54	81	81	81
257	4	13	32	32	48	48	48	72	72	108	108
258	1	3	8	8	12	12	12	18	18	18	27
267	2	7	16	16	24	24	24	24	36	54	54
268	3	10	24	24	36	36	36	36	54	54	81
278	2	7	16	16	24	24	24	24	24	36	54
347	2	7	16	16	16	24	36	36	36	54	54
348	1	3	8	8	8	12	18	18	18	18	27
356	1	3	8	8	8	12	12	18	27	27	27
357	2	7	16	16	16	24	24	36	36	54	54
358	3	10	24	24	24	36	36	54	54	54	81
367	1	3	8	8	8	12	12	12	18	27	27
368	1	3	8	8	8	12	12	12	18	18	27
458	1	3	8	8	8	8	12	18	18	18	27
468	4	13	32	32	32	32	48	48	72	72	108
478	1	3	8	8	8	8	12	12	12	18	27
568	1	3	8	8	8	8	8	12	18	18	27

In the above analysis it has been assumed that while the relative frequencies of tetrads may differ, within each type of tetrad the frequencies of strands that are recognizable as crossovers in any specified regions are the same as when recurrence is random. This is necessarily true when sister strands do not cross over, but otherwise need not be true for all tetrads. Hence substitutions differing from those given above might be possible.

The results of non-random recurrence can also be tested by means of the formulas for cases 3 and 4. For the frequency of tetrads of rank 0, formula 6 will be sufficient, at any rate for purposes of illustration.

An increase of regressives, or of progressives at the expense of digressives, will decrease t_{1-2} and t_{-1+2} and will increase t_{1+2} and t_{-1-2}. This will lessen the value of the term in a_2 and consequently of y_0; hence there will be even more negative tetrads if sister strands cross over. An increase of digressives, or of progressives at the expense of regressives, will increase t_{1-2} and t_{-1+2} and will decrease t_{1+2} and t_{-1-2}. This will increase the value of the term in a_2 and if the increase is sufficient the negative frequency of non-crossover tetrads will disappear.

Other classes can be evaluated in the same way; and the method is essentially equivalent to the analysis already given. For some purposes it may be preferable, since it can be used more systematically; for example, the t's may be made to vary according to some rule, such as that $t_{1+2+3} = t_{1+2}t_{2+3}$, and the correctness of the rule can thus be tested.

The analysis of the Xple data is supported by the results of applying the multiple-strand theory to a cross involving the genes *sc ec cv ct v s f car bb*, which cover practically the entire crossover map of the X chromosome (table 8). The observed frequencies (column A) are from an experiment made by DR. C. B. BRIDGES, who kindly placed them at my disposal before they were published (MORGAN, BRIDGES, and SCHULTZ 1935).

Negative values appear in the non-crossover tetrads as well as some of those of rank 1 when sister strands are allowed to cross over beyond the first two regions.

Here the negative values cannot be eliminated so simply as in the X-ple cross.

The analysis is further supported by the application of the theory to other data, both sex-linked and autosomal, in *Drosophila melanogaster* and to sex-linked data in *D. virilis*. These results will be published elsewhere.

The maximum amount of recombination between two linked genes

In most organisms the amount of recombination between two linked genes approaches 50 percent as an upper limit as the intermediate distance lengthens. This relation is a corollary of equation 2b, which can be written

$$(a_0+a_2+a_4+\cdots)-(a_1+a_3+a_5+\cdots)=2^r\mathrm{X}.$$

If the assumptions on which the equation is based are correct, then $2^r\mathrm{X}$ cannot be negative. Hence $(a_0+a_2+a_4+\cdots)\geqq(a_1+a_3+a_5+\cdots)$; that is, the apparent non-crossovers (classes of 0 and even rank) will equal or exceed the apparent crossovers (classes of odd rank).

This relation can be deduced directly from the set of equations on page 163; as in fact it has been deduced by EMERSON and RHOADES (1933) from the table of BELLING (1931) which corresponds to a special case of these equations. In each vertical column of the equations the coefficients of the

x's are the successive terms of the expansion $(p+q)^r$, where $q=1-p$. If $p=1/2$, the sum of the alternate terms is $1/2$; and in any column (except the x_0 column) the strands with no crossings over or with an even number will constitute half the total. These strands together with those in the x_0 column make up the apparent non-crossovers; hence the observed recombination frequency will be less than 50 percent. As the chromosome lengthens genetically, the x_0 class will decrease and approach 0 and the proportion of recombination between the end genes will approach 50 percent as an upper limit.

A more general relation can be obtained if the equations are multiplied respectively by $1, -1, 1, \cdots (-1)^n$ and added together. The result is the equation

$$(a_0+a_2+a_4+\cdots)-(a_1+a_3+a_5+\cdots)$$
$$=x_0+(q-p)x_1+(q-p)^2x_2+\cdots+(q-p)^nx_n. \qquad (14)$$

If $p=1/2$, $q-p=0$, and we have the case just discussed.

If $p<1/2$, the right-hand side of the equation will remain greater than 0, and the recombination frequency between the end genes will remain less than 50 percent as n increases.

If $p>1/2$, the terms in $(q-p)$ will be positive if the exponent is even, negative if it is odd. Hence their sum will be increased by the terms with even exponents, decreased by those with odd exponents. The precise nature of the result will therefore depend on the number of x's and their relative sizes; that is, on the length of the chromosome and the coincidence. This situation would result if more than two chromatids crossed over at one level or within a region.

The relations just deduced are only part of still more general relations. For in the equations on page 163, a_0 may represent the frequency of any class, not necessarily the non-crossovers. It follows that if $p=1/2$, the sum of the crossovers in any specified region or regions and in 0, 2, 4, $\cdots$ additional regions will exceed the sum of the crossovers in the specified region or regions and in 1, 3, 5, $\cdots$ additional regions. This relation holds for *Drosophila melanogaster* and also for *D. virilis* (WEINSTEIN, unpublished data involving the X chromosome from sepia to rugose, a distance of about 100 units.

The minimum distance within which double crossing over occurs

An upper limit can be set to the possible frequency of crossing over between sister strands by the length of the shortest distance between adjacent levels of crossing over in a chromatid. In the X chromosome of *D. melanogaster*, this is about 14 units. Since the frequency of tetrad crossing over in such a region cannot exceed 1.00, the chance of detecting a

crossing over, being the ratio of the observed to the actual frequency, cannot be less than 0.14. But the chance of detecting a crossing over in a tetrad is composed of two factors: the chance of recovering a crossover chromosome and the chance of recognizing it when recovered. The first factor is 1/2 (provided that only two strands cross over within the region); hence the second is not less than 0.28. That is, crossover strands can be recognized in at least 0.28 of cases, and sister strands cannot cross over in more than 0.72 of cases.

It might be possible to draw a further conclusion in some cases. If every tetrad were a crossover in the region, the amount of crossing over in the region could not exceed 33 1/3 percent if sister strands crossed over and 50 percent if they did not. Thus single crossing over exceeding 33 1/3 percent within a region where no double crossing over occurred might be an indication that there was no crossing over between sister strands.

COINCIDENCE AND INTERFERENCE

The coincidence of two regions may be expressed as

$$\frac{D/N}{A/N\ B/N} = \frac{DN}{AB},$$

where N is the total number of individuals, A the number of crossovers in the first region, B in the second region, and D in both regions simultaneously. The regions whose coincidence is being measured will be referred to as nodal regions; the points of crossing over as nodes. The distance between the nodes will be termed the internode, and the distance between the nodal regions will be termed the intermediate region.

Coincidence may be of several types. In the type originally defined, A, B, and D include all individuals that are crossovers in the nodal regions, regardless of whether they are also crossovers in other regions. They may be termed inclusive totals, and coincidence so calculated inclusive coincidence. Inclusive coincidence, which is a measure of interference, increases as the internode lengthens (MULLER 1916).

If crossovers in the intermediate region are excluded from D, the resultant coincidence may be termed select; it measures the frequencies of internodes of different lengths (WEINSTEIN 1918).

If crossovers in the intermediate region are excluded from A, B, D, and N, the resultant coincidence has been termed partial (MULLER 1925). The concept of partial coincidence may be extended by excluding regions other than the intermediate ones; in the extreme case all regions may be excluded except those whose coincidence is being measured. This extreme type may be termed exclusive coincidence.

All the above types of coincidence as ordinarily measured are based on the observed frequencies of chromatids. They may therefore be termed chromatid or strand coincidence. Coincidence might also be based on true chromatid frequencies, or on tetrad frequencies. Tetrad coincidence might be based merely on the levels of crossing over, regardless of which strands are involved; or it might be calculated separately for regressive, progressive, and digressive crossing over; and further distinctions are possible according to whether the exchanges are between lateral or diagonal, homologous or sister strands.

Inclusive coincidence

If recurrence is random, inclusive coincidence for tetrads or for the true frequencies of strands is equal to the strand coincidence as ordinarily calculated. For if X_A, X_B and X_D denote respectively the inclusive frequencies of tetrads that are crossovers in the first nodal region, in the second nodal region, and in both nodal regions, then strand coincidence whether based on observed or true values is $p_A\ p_B\ X_D\ N/p_A\ X_A\ p_B\ X_B = X_D\ N/X_A\ X_B$, which is the tetrad coincidence. This invariance holds regardless of whether or not sister strands cross over, and regardless of whether the p's are the same or different in different regions.

But if recurrence is not random, strand coincidence is

$$t_{A+B}\ p_A\ p_B\ X_D\ N/p_A\ X_A\ p_B\ X_B;$$

hence

$$\text{inclusive tetrad coincidence} = \frac{\text{inclusive chromatid coincidence}}{t_{A+B}} \qquad (15)$$

If random recurrence is altered by an increase of progressives at the expense of digressives, or by an increase of regressives, $t_{A+B} > 1$, and tetrad coincidence will be less than the observed value. If random recurrence is altered by an increase of progressives at the expense of regressives, or by an increase of digressives, $t_{A+B} < 1$, and tetrad coincidence will be greater than the observed value.

Only those deviations from random recurrence need be considered that yield the observed frequencies of chromatids: for example, those indicated in equations 8–13 and in table 7. These deviations will not alter the frequency of recognizable crossing over in any one region, and hence will not alter the denominator of the coincidence fraction. Their effect on coincidence will result from an alteration of the number of double crossover tetrads and hence of the numerator of the fraction.

The substitutions indicated in equations 9, 11, and 12 would not alter the total number of tetrads that are crossovers in both regions involved and hence would not affect the coincidence. This applies to equation 11

only if the rank-2 regressive involves both nodal regions. But a change would result from the substitutions indicated in the other equations. Since, on random recurrence without sister-strand crossing over, the progressives constitute one-half of rank-2 tetrads, the regressives and digressives one quarter each, the substitutions in equation 10 might increase or decrease tetrad coincidence by as much as 25 percent. A similar decrease might result from the elimination of digressives indicated in equation 8. The reverse substitution in this equation would increase tetrad coincidence by an amount dependent on the number of singles available. Thus the coincidence of regions 1 and 6, which is $912 \cdot 28239/4036 \cdot 6442 = 1.0$, could be increased to 2.9 if all the 1744 singles in region 1 and an equal number in region 6 were replaced by 1744 digressives and 1744 non-crossovers. Similarly, the coincidence of regions 2 and 6 could be increased from 1.0 to 2.5 if the 1998 singles in region 6 and an equal number in region 2 were replaced by digressives and non-crossovers.

Not all the coincidence values could be altered simultaneously. Thus if all the 1998 singles in region 6 were used to raise the 2, 6 coincidence, there would be none left to raise the coincidence of 1 and 6. Again, only 1709 digressives could be added or eliminated by equation 8 since there are only 1709 non-crossovers. If the changes are distributed among the various classes, the increase or decrease in any one class would be much less than indicated above.

Similar procedures with similar results apply if there is crossing over between sister strands. The distribution of tetrads in table 7 would increase coincidence since it adds 5316 tetrads to rank 2. The increase would be negligible for all values involving region 6, for the additional doubles are at the expense of singles, of which there are only 33 in region 6 (table 4, column I); but other values might be considerably affected. The increases could be minimized if the additional tetrads were distributed among all classes; but they could not be counteracted by substitutions since there are no non-crossovers at whose expense these could be made.

It is perhaps unlikely that inclusive tetrad coincidence differs greatly from the observed value, for the following reasons: (1) Great deviations are brought about only by restricting substitutions of tetrads to one or a few classes, with the result that two adjacent regions have very different coincidences with the same region. (2) An increase of coincidence to more than 1 would imply that crossing over in one region is helped by that in another. (3) A decrease of inclusive coincidence for widely separated regions to less than 1 is not consistent with the mutual independence that might be expected from such regions. But we do not know how widely separated two regions must be to achieve independence; and in our present state of knowledge none of these reasons is conclusive.

Select coincidence

If $X_d, X_{d+1}, X_{d+2}, \cdots X_{d+n}$ are the frequencies of tetrads that are crossovers in both nodal regions and also at 0, 1, 2, $\cdots$ n levels in the intermediate region, then if recurrence is random and p constant,

$$\text{select chromatid coincidence} = \frac{p^2[X_d + qX_{d+1} + q^2X_{d+2} + \cdots + q^nX_{d+n}]N}{p\,X_A \quad p\,X_B}$$

$$= \text{select tetrad coincidence} + \frac{[qX_{d+1} + q^2X_{d+2} + \cdots + q^nX_{d+n}]N}{X_A\,X_B} \qquad (16)$$

Hence select coincidence for tetrads is less than the true value for chromatids, which in turn is less than the observed value since q for the true value is less than for the observed value. These relations hold if the p's differ, but not necessarily if recurrence is not random.

As the intermediate region lengthens, there will ultimately be a decrease in the frequency of tetrads that are not crossovers in it, and consequently a decrease of select tetrad coincidence. Select coincidence for strands will also ultimately decrease if recurrence is random, and even if it is not random except on rather special assumptions.

Partial coincidence

Partial coincidence for tetrads will in general differ from the observed value; for when tetrad frequencies are replaced by the corresponding strand frequencies, the changes in the numerator and denominator do not necessarily compensate for each other. The same is true when non-intermediate regions or all non-modal regions are excluded.

A detailed discussion of coincidence in *D. melanogaster* and *D. virilis* will be published separately.

NON-DISJUNCTION AND ATTACHED X'S

The multiple-strand method can be applied to cases of non-disjunction and attached X's if allowance is made for the fact that two chromatids are recovered instead of one and that they are not necessarily a random pair. The situation is clearest in attached X's, where the genes in the attached strands tend to remain together, the tendency being absolute at the point of attachment and decreasing distally because of crossing over with strands of the other attached pair.

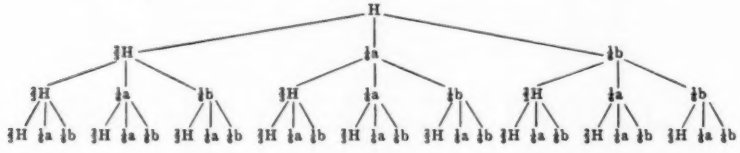
It will be best therefore to begin at the point of attachment; and if we do this, the strands resulting from tetrads of any given rank can be deduced as follows.

Crossing over between the spindle fibre and the first heterozygous pair

of genes might occur in three ways: (1) between strands attached to each other (these are homologous); (2) between sister strands; and (3) between a strand and its homologue in the other attached pair. Types 1 and 2 will leave each attached pair heterozygous; type 3 will result in homozygosis in each pair. If any two chromatids are equally likely to cross over, the three types will occur with equal frequency, and homozygosis will be produced in 1/3 of the cases.

If the first crossing over leaves each attached pair of chromatids heterozygous, then crossing over in the next region can occur in the same three ways, with the same results. If the first crossing over has produced homozygosis, the next crossing over can occur in two ways: (1) between strands attached to each other (these are now sister strands), (2) between strands of different attached pairs (these are now all homologous). If it is a matter of chance which strands cross over, type 1 will occur in 1/3 of cases, type 2 in 2/3.

This procedure can be continued for the entire length of the chromosome. It may be represented by the following diagram, in which a represents homozygosis for one strand, b for the other, and H heterozygosis.



The chance that a tetrad of any rank will give rise to a particular type of offspring can be obtained by multiplying the appropriate fractions. For example, among offspring derived from tetrads of rank 3, there will be $(2/3)^3$ that are heterozygous throughout and $(1/6)^3$ that are homozygous for strand a throughout.

If crossing over does not occur between sister strands, but association of strands is otherwise random, the procedure must be modified to conform with the following diagram:

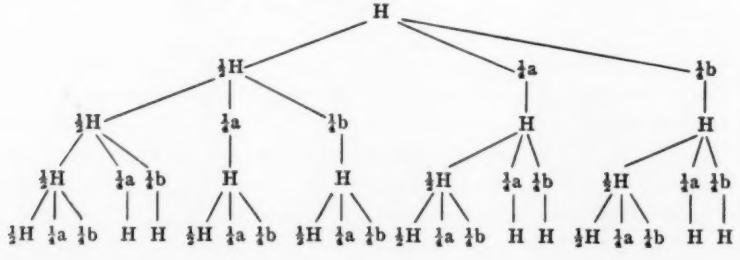


TABLE 9*

Calculated and observed frequencies of daughters of attached-X females
(Observed frequencies from Sturtevant 1931)

I — + / *sc ec cv cl v g f*

	Obs.	Calc.
+	614	613
sc	27	19
sc ec	36	29
sc ec cv	18	27
sc ec cv cl	47	45
sc ec cv cl v	16	25
sc ec cv cl v g	9	15
sc ec cv cl v g f	5	9
ec	1	0.2
ec cv	2	0.4
ec cv cl	4	4
ec cv cl v	5	6
ec cv cl v g	9	6
ec cv cl v g f	3	6
cv	2	0.4
cv cl	7	4
cv cl v	12	8
cv cl v g	10	9
cv cl v g f	3	6
cl	4	1
cl v	4	5
cl v g	6	6
cl v g f	5	6
v	6	3
v g	8	10
v g f	9	7
g	1	2
g f	4	4
f	1	1
sc v g	2	0.2
Total	880	

II — *ec cv cl v g f* / *sc*

	Obs.	Calc.
+	557	578
sc	198	210
ec	34	36
ec cv	29	35
ec cv cl	54	61
ec cv cl v	32	38
ec cv cl v g	24	26
ec cv cl v g f	6	19
cv cl	9	5
cv cl v	19	10
cv cl v g	7	12
cv cl v g f	8	8
cl	0	1.6
cl v	15	6
cl v g	10	8
cl v g f	7	8
v	9	4
v g	18	12
v g f	21	9
g	3	2
g f	17	4
f	2	1.1
ec v g	1	0.2
ec cv g f	2	0.1
ec cv cl g f	2	0.5
ec cv cl f	1	0.2
sc v g	1	0.6
sc v g f	3	0.3
sc g	1	0.2
sc g f	4	0.7
sc f	1	0.2
sc cl v g f	1	0
Total	1096	

III — *g f* / *sc ec cv cl v*

	Obs.	Calc.
+	515	534
sc	16	19
sc ec	20	28
sc ec cv	22	27
sc ec cv cl	28	44
sc ec cv cl v	39	48
g	56	49
g f	38	32
ec cv	2	0.4
ec cv cl	7	4
ec cv cl v	28	17
cv	1	0.4
cv cl	5	4
cv cl v	28	23
cl	2	1.3
cl v	30	17
v	27	20
f	2	1.1
sc v	2	0.5
sc ec v	1	0.2
sc g	1	0.3
sc ec g f	3	0.2
Total	873	

IV — *ec cv* / *sc* — *g f* / *cl v*

	Obs.	Calc.
+	308	318
sc	74	61
sc cl	39	37
sc cl v	36	40
ec	28	46
ec cv	83	65
ec cv g	9	18
ec cv g f	5	13
cl	17	8
cl v	37	48
cv	6	10
cv g	7	8
cv g f	4	5
g	21	15
g f	17	14
v	25	16
f	2	0.7
ec g	1	0.1
ec g f	1	0.2
ec cv f	1	0.1
sc g	1	0.5
sc g f	1	0.5
sc f	1	0.1
sc cl g	1	0.0
Total	725	

V — *cl v f* / *sc ec cv g*

+	349	362
sc	22	16
sc ec	15	23
sc ec cv	56	80
sc ec cv g	11	21
cl	46	44
cl v	68	66
cl v f	15	23
ec cv	17	8
ec cv g	10	10
cv	14	10
cv g	15	13
v	15	11
v f	7	6
g	44	29
f	14	4
sc g	3	0.5
sc ec g	2	0.3
sc cl v	1	0.1
sc v f	1	0.2
sc f	1	0.1
sc ec v f	1	0.1
sc ec f	1	0.2
Total	728	

VI — *ec* / *sc cv cl* — *v f* / *g*

+	386	397
sc	62	49
sc cv	20	28
sc cv cl	37	72
sc cv cl g	9	26
ec	102	109
ec v	43	54
ec v f	13	16
cv	2	0.8
cv cl	32	22
cv cl g	28	29
cl	14	6
cl g	12	13
v	61	43
v f	24	20
g	39	23
f	11	4
cv g	1	0
sc g	5	1
sc v	6	0.6
sc v f	4	0.3
sc f	1	0.3
ec g	4	1
Total	916	

VII — *ec cl v* / *sc cv* — *f* / *g*

+	352	398
sc	69	49
sc cv	90	100
sc cv g	12	26
ec	65	58
ec cl	48	50
ec cl v	54	53
ec cl v f	4	16
cv	31	23
cv g	34	29
cl	14	6
cl v	44	29
cl v f	15	13
v	15	14
v f	5	7
g	47	36
f	3	4
sc g	2	1
ec v	3	0.3
ec cl f	1	0.5
sc cl v	1	0.0
sc v f	2	0.3
sc f	1	0.3
ec g	2	0.6
ec cl g	1	0.4
ec cv	1	0
Total	916	

VIII — *cv* / *sc ec cl v* — *g* / *f*

+	413	433
sc	35	20
sc ec	59	59
sc ec cl	38	47
sc ec cl v	21	42
sc ec cl v f	2	9
cv	121	125
cv g	40	55
ec	8	0.6
ec cl	7	4
ec cl v	17	12
ec cl v f	3	6
cl	10	6
cl v	41	30
cl v f	21	13
g	43	37
v	17	14
v f	19	7
f	4	5
sc v f	1	0.2
sc ec v	1	0.4
sc cv g	1	0.1
sc g	1	0.6
sc ec g	3	0.6
sc ec cl g	1	0.3
ec g	1	0.0
Total	928	

* This table includes all calculated frequencies of 1 or more; and, where the observed frequency is not 0, every calculated frequency of less than 1. Calculated figures are given to the nearest unit; except those of 1 or less, which are given to the nearest 0.1.

In either of the above cases, if recurrence is not random, the frequency of each class obtained from a tetrad of a given kind will not be the simple product of the fractions in the table, but this product multiplied by a factor which, in the most general case, will differ according to the rank of the tetrad, the regions involved, and the nature of the crossings over: whether homologous or sister-strand; lateral or diagonal; regressive, progressive, or digressive.

Thus if the frequencies of tetrads are known, the frequencies of genotypes and phenotypes among the offspring can be calculated. In table 9 are given the frequencies of phenotypes expected among offspring of attached-X females heterozygous for *sc ec cv ct v g f*, on the assumption that sister strands do not cross over but association of chromatids is otherwise random. The tetrad frequencies in the column headed $p=1/2$ in table 4 have been used as a basis; but since they do not include crossing over between the spindle fibre and forked, a correction has been applied by taking into account what proportion of each class must also have been crossovers to the right of forked. This correction is based on a cross involving the loci *y bi cv ct v g B bb*, the unpublished data of which were kindly placed at my disposal by DR. C. B. BRIDGES in 1932. The crossover values in this cross agree closely with those in the X-ple cross (MORGAN, BRIDGES and SCHULTZ 1933).

The calculations have been made for the eight types of heterozygous mothers that gave the greatest number of offspring in the experiments of STURTEVANT (1931), and STURTEVANT's actual counts are included for comparison. The frequencies are on the whole in agreement. The discrepancies are probably due at least in part to the small counts and to differential viability; there may also have been differences in proportions of crossing over between the attached-X stock and those on which the calculated values are based.

A comparison can also be made between calculated and observed frequencies of offspring heterozygous for all the genes involved. The calculated value is 39.9 on the basis of table 4 (corrected) and 40.9 on the basis of table 8. The value observed by STURTEVANT (1931) is 34.5; and from the data given by BEADLE and EMERSON (1935, table 2) it appears that among 1478 offspring whose genetic constitutions were tested, 668 were heterozygous for all genes from scute to forked inclusive, this being a proportion of 45.2. The calculated values are almost precisely half way between the observed values. It should be noted also that STURTEVANT's figure is based on tests of only 383 wild-type daughters; and that the proportions of crossing over in the attached-X stock used by BEADLE and EMERSON were somewhat different from those of the stocks on which the calculated values are based.

The proportion of completely heterozygous individuals in attached X's, is equal to $x_0+qx_1+q^2x_2+q^3x_3+\cdots+q^nx_n$ provided that at any level of crossing over only two strands are involved; hence it is precisely equal to the proportion of non-crossovers in the same region among offspring of females with unattached X's. The variations in the frequencies of x's and p's compensate for one another, and the result is therefore independent of the value of p. Nevertheless a figure obtained on the assumption of crossing over between sister strands would not be significant despite its agreement with observation; for the tetrad frequencies on which it would be based are incorrect, involving as they do negative values. The agreement would therefore be purely formal since it would be due to compensating errors.

The proportion of homozygosis for each gene can also be calculated. This is done by adding the proportions of the appropriate classes in the diagrams on page 182. If $p=1/2$, the sum is

$$\tfrac{1}{4}x_1+\tfrac{1}{8}x_2+\tfrac{3}{16}x_3+\tfrac{5}{32}x_4+\cdots$$

where the x's are the frequencies of tetrads that are crossovers at 1, 2, 3, 4, $\cdots$ levels between the point of attachment and the gene in question. Values have been calculated on the basis of the $p=1/2$ columns of table 4 (corrected for crossing over between *f* and *bb*) and of table 8. These, together with observed values, are given in table 10 and illustrated in figure 4.

The solid curves in figure 4 illustrate homozygosis plotted against actual map distance in each cross. The two lines coincide almost completely from the spindle fibre to about *v*; beyond *v* the curve based on table 4 rises above that based on table 8.

The total map distance is almost exactly the same in the two crosses; but corresponding regions do not always have the same length. Hence, while the ends of the curves lie in the same perpendicular, the other corresponding loci do not. In order to facilitate the comparison of corresponding genes, the curves have been redrawn so that the abscissa of each gene is the average of the values in the two crosses. The results are the dash line for the data of table 4 and the dot-and-dash line for those of table 8.

The theoretical and observed results are of the same general type, rising from nearly 0 at the proximal end to above 16 2/3 (the value expected on pure chance) at the distal end. The greatest discrepancies are the low values observed by EMERSON and BEADLE in the region from *car* to *ct;* these are undoubtedly due to the fact that there was less crossing over in the attached X stock used by EMERSON and BEADLE than in the stocks of tables 4 and 8. Differences in crossing over and in coincidence may also account in part for the discrepancies in *ct* and more distal genes; but differ-

ential viability is suggested by the low value of *cv* as compared with *ct* and of *sc* as compared with *y* in some of the data.

The theoretical curve based on table 4 and on the *y bi cv ct v g B bb* cross was exhibited at the Sixth International Congress of Genetics and before the Genetics Society of America in 1932. Homozygosis when sister strands do not cross over was independently calculated by SAX (1932) and

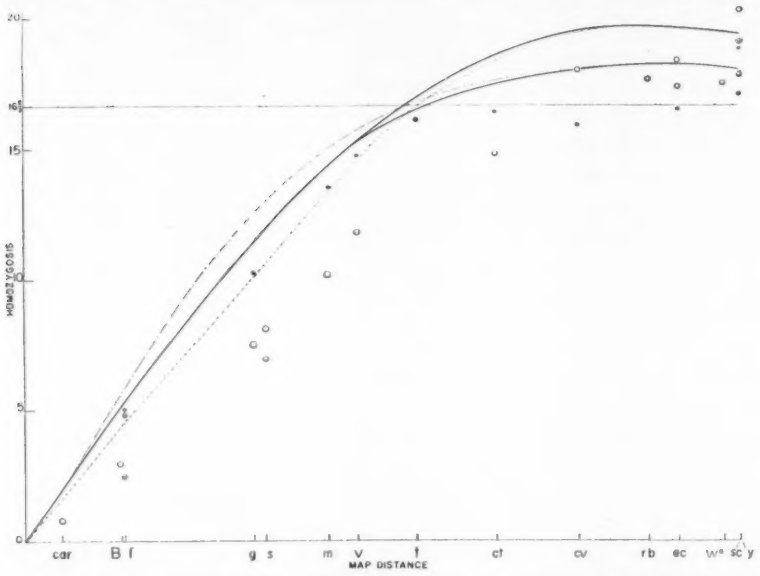


FIGURE 4. Proportions of homozygosis for recessive genes in offspring of heterozygous attached-X females. The abscissa indicates distance from the spindle fibre. The curves are drawn through the theoretical values given in table 10. The observed values in table 10 are also indicated; those from ANDERSON, L. V. MORGAN, and STURTEVANT by dots enclosed in circles; those from RHOADES by concentric circles; those from EMERSON and BEADLE by simple circles.

subsequently by BELLING (1933), KIKKAWA (1933)[2] and MATHER (1935); the data on which these calculations were based did not include the entire length of the X chromosome. BEADLE and EMERSON (1935) have obtained a similar curve from an analysis of crossing over in the attached X's themselves.

[2] KIKKAWA's previous (1932) method was, as he himself pointed out, incorrect because the highest frequencies of homozygosis yielded by it were distinctly less than 16 2/3 percent. KIKKAWA in 1932 stated that the writer's theory as embodied in formula 2 of the present paper would not allow for *aaa/aba* individuals. This objection is answered by the analysis given above, which shows that such individuals are expected and with a frequency corresponding to the one observed experimentally. KIKKAWA's objection was due to a misinterpretation of the writer's theory; special cases of the theory have since been used by KIKKAWA himself.

The agreement of a theory with observation is of course not significant unless other theories agree less closely. It is therefore necessary to consider how the curve would be modified if the assumptions on which it was calculated were altered.

For regions near the point of attachment the curve is a straight line, the homozygosis being half the map distance from the spindle fibre. This

TABLE 10*

Percentages of homozygosis in daughters of attached-X females.

GENE	CALCULATED		OBSERVED			
	TABLE 4 p=1/2 CORRECTED FOR CROSSING OVER BETWEEN *f* AND *bb*	TABLE 8 p=1/2	ANDERSON 1925 L. V. MORGAN 1925 STURTEVANT 1931 SUMMARIZED BY STURTEVANT	RHOADES 1931	EMERSON AND BEADLE 1933	BEADLE 1935
car		2.2				0.8
B			4.1		3.0	
f	4.6	5.9	5.1	4.9		2.5
g	10.1		10.3		7.5	
s		13.0			8.1	7.0
m			13.5		10.2	
v	14.6	15.8	14.8		11.9	11.8
lz			14.9			
t			16.1			
ct	18.4	17.6	16.4			14.8
cv	19.5	18.1	15.9			18.0
rb				17.6		
ec	19.6	18.3	16.6	17.3		18.4
w or w^a			16.5 (*w*)	17.5 (w^a)		
sc	19.4	18.0	17.1	17.9		20.3
y			19.0	19.2		

* This table includes all the data hitherto published. The proportions headed RHOADES (1931), and EMERSON and BEADLE (1933), have been calculated by the writer from the total counts of these workers. The homozygosis values calculated by RHOADES differ slightly from those recorded here because he excluded some of his data.

In the experiment of EMERSON and BEADLE (1933) the value marked *B* is the average for Bar and its normal allelomorph.

would be expected on random association without sister strand crossing over; because homozygosis for a given gene is 1/4 of the frequency of tetrads of rank 1, while the map distance is 1/2, and there are few if any tetrads of higher rank. If sister strands cross over freely, homozygosis is 1/6 and map distance 1/3 of rank-1 tetrads; so that the agreement is equally good and this part of the curve cannot be used as evidence for or against sister-strand crossing over. But the distal part of the curve, as has been pointed out by the writer (WEINSTEIN 1932c) supplies the necessary evidence; for if sister strands crossed over, the proportion of homozygosis (as can be seen from the first diagram on page 182) would be

$$\tfrac{1}{6}x_1+\tfrac{1}{6}x_2+\tfrac{1}{6}x_3+\cdots+\tfrac{1}{6}x_n$$

so that even if all tetrads were crossovers, the amount of homozygosis would not exceed one-sixth. The fact that both calculated and experimental curves rise above this level shows that crossing over does not occur freely between sister strands.

The curve also proves that only two of the four strands cross over at any level. For if all four always crossed over, no homozygosis would be produced at all; and if all four sometimes crossed over, the homozygosis in the proximal part of the curve would not be half the map distance but less. This is true regardless of whether crossing over occurs between sister strands.

The curve can also be used to test whether recurrence is random. That the proportion of homozygosis depends on the nature of recurrence can be seen from the fact that in tetrads of rank 2 homozygosis of genes distal to both exchanges results not from regressives or digressives but only from some of the progressives. If there is no crossing over between sister strands, homozygosis is produced only by those progressives in which the proximal crossing over is lateral (the distal crossing over must then be lateral also); this type is illustrated in figure 2B. The terms lateral and diagonal are applied according to the relative positions of the strands in the region of crossing over, and do not necessarily describe their relation at the proximal end.

If recurrence is random, these progressives constitute one-half of all progressives or one quarter of all rank-2 tetrads.[3] That is, the same proportion of rank-2 progressives as of single crossover tetrads produce homozygosis; while regressives, digressives, and non-crossovers do not produce it at all.

The substitutions indicated in equations 8–13 and table 7 can be tested with respect to their effect on homozygosis for scute with the aid of the following table.

TABLE 11

Tetrad frequencies for the X chromosome (random recurrence, no sister-strand crossing over)

RANK	0	1	2 REGRESSIVE	2 PROGRESSIVE	2 DIGRESSIVE	3	4
Table 4 (corrected to include crossing over between *f* and *bb*)	557	15678	2755	5510	2755	888	96
Table 8	904	7824	1730	3460	1730	488	

Equation 8. The replacement of non-crossovers and digressives by singles would increase homozygosis. In the corrected Xple figures, since

[3] All these proportions must be divided by 2 when homozygosis for the recessive gene only is considered.

there are only 557 non-crossover tetrads there could be not more than 1114 additional singles, of which 278.5 would produce homozygosis for scute; this would raise the proportion from 19.4 to 20.4—not so good an agreement with the observed values for *sc* and *y*.

The reverse substitution (non-crossovers and digressives for singles) could have a much greater effect. If all of the 15678 singles were replaced, homozygosis for *scute* would fall to 5.5, an impossibly low figure. A reduction of homozygosis to 16.7 would require the replacement of 1/5 of the singles; this is highly improbable, since most of the observed values for *sc* and *y* differ significantly from 16.7.

On the basis of table 8, the highest value would be 20.8, the lowest 5.9; and a decrease to 16.7 would involve replacing 1/9 of the singles.

Equation 9. The progressives could be doubled or eliminated; and homozygosis would fall as low as 14.6 or rise as high as 24.2 on the basis of table 4 (corrected). The corresponding values on the basis of table 8 would be 12.6 and 23.4. Hence only a small part of the substitutions would be possible.

Equation 10. This would not alter homozygosis, since the effectiveness of a progressive is equivalent to that of a single.

Equation 11. Homozygosis would remain unaltered because a non-progressive tetrad of rank 3 has the same effect as a single. This follows from the fact that in the rank-3 tetrad two of the crossings over are regressive with respect to each other and neutralize each other; the third crossing over effects homozygosis if it is diagonal but not if it is lateral.

If sister strands cross over freely, homozygosis results from 1/3 of the tetrads of rank 1 and 1/2 of the progressives of rank 2, but not from regressives or digressives of rank 2 or from non-crossovers (see footnote 3). Since homozygosis calculated on free sister-strand crossing over and random recurrence is too low, only those deviations from random recurrence are possible that raise the calculated proportion to the observed level.

The method by which the negative frequency of non-crossovers was eliminated (table 7) consisted in decreasing the number of tetrads of rank 1 by 10632 and increasing the digressives by 5316. This would decrease the proportion of homozygosis for distal genes and is therefore impossible.

Of the substitutions indicated in equations 8, 11, 12, and 13 the only ones that would increase homozygosis are the replacement of the left-hand by the right-hand side of equations 8 and 12. But singles cannot be increased by the substitutions indicated in equation 8, since there are no non-crossovers at whose expense this could be accomplished; hence the only substitution that remains is of progressives for regressives and digressives (equation 12). To test this completely the figures in table 7

do not suffice, since they include only the region from *forked* to *scute;* but they can be considered as roughly applicable to the region from the point of attachment to *crossveinless* or *echinus*, which is about as long as that from *forked* to *scute*. On this basis there might be as many as 10820 additional progressives; and the proportion of homozygosis due to singles and progressives might be increased to as much as 1/28239 (1/6 4698+1/4 22016) = 22.2—too high a figure, so that only part of the substitution is possible.

POLYPLOIDS

Triploids

In triploids there is evidence that only two of the six strands cross over at any level (BRIDGES and ANDERSON 1925, REDFIELD 1930). Hence among the strands emerging from a hexad which is a crossover at a given level, the proportion that are recognizable crossovers at that level is the product of 1/3 by the chance of recognizing a crossover strand once it is recovered. This chance depends on two factors: (1) whether all three chromosomes are distinguishable from each other or two are indistinguishable, and (2) whether crossing over takes place between sister strands. The results for four possible cases are as follows:

	CROSSING OVER BETWEEN SISTER STRANDS AS FREQUENT AS ON CHANCE	NO CROSSING OVER BETWEEN SISTER STRANDS
All 3 chromosomes distinguishable	1/3(4/5) = 4/15	1/3
2 chromosomes indistinguishable	1/3(8/15) = 8/45	1/3(8/12) = 2/9

These fractions represent the chances of detecting a crossing over at one level if one chromatid is recovered ("regular" offspring). If two chromatids are recovered ("exceptional" offspring), then p for hexads is the product of the chance that *at least* one is a crossover by the chance of recognizing a crossover strand once it is obtained provided that the two chromatids are recovered at random.

BRIDGES and ANDERSON (1925) showed that a chromosome may cross over with different chromosomes at different levels; and they concluded that synapsis involves all three chromosomes equally throughout their length. On this theory recurrence would be random for all six chromatids, except that sister-strand crossing over may be excluded. But when hexad frequencies are calculated on this basis, negative values are encountered (WEINSTEIN 1932c).

In table 12 are given the hexad frequencies calculated for the cross of REDFIELD (1930, table 1, broods 1 and 2 combined), which covers about half of the third chromosome. Here all three chromosomes are marked;

hence formula 2 can be applied with $p = 4/15$ and $p = 1/3$. When $p = 4/15$, the largest negative value (-135) is 1.6 times its standard error. When $p = 1/3$, the negative value (-30) is 0.6 times its standard error. The results are not conclusive; still they suggest that with longer stretches of chromosome significant negative frequencies would appear.

TABLE 12

Crossing over in triploids of constitution + *D ma Sb* + / *se Mh* + + *H* / *h* + *cu bx e*[a]

REGIONS OF CROSSING OVER	OBSERVED FREQUENCIES REDFIELD 1930 n = 1030	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE p=1/2	p=1/3	p=4/15
0	565	272	127	101
1	87	100	63	17
2	171	226	183	97
3	70	56	−30	−135
4	47	48	21	−7
12	22	76	144	193
13	11	36	63	77
14	8	20	18	−4
23	27	104	225	341
24	12	40	72	91
34	6	20	36	46
123	1	8	27	53
124	2	16	54	105
134	1	8	27	53

Negative values are in fact obtained for the cross covering almost the entire length of the third chromosome given in REDFIELD's table 5. Here only one of the chromosomes is marked, hence $p = 2/9$. And a further modification of procedure is necessitated by the fact that since there are two strands of one kind and one of the other, the chances of recognizing crossing over at different levels are not independent, even though recurrence be random. It becomes necessary therefore to use equations like those in case 3. The analysis need not be given in detail because a similar one has been made by other workers (MATHER, 1933, 1935; KIKKAWA, 1934; see KIKKAWA for the equations). These negative frequencies are not in themselves significant; but they point in the same direction as those in the previous cross.

These results suggest that, for considerable distances, only two of the

chromosomes conjugate while the third goes unmated to either pole. On this assumption, the unmated chromosome region can be neglected; for the conjugating chromosomes form what is essentially a tetrad, and the chromosome recovered in regular offspring is always one of those that conjugate. Hence the data in table 12 can be treated as in diploids; that is, formula 2 can be applied with $p=1/3$ if sister strands cross over and $p=1/2$ if they do not. When $p=1/3$, as we have seen, some negative values are encountered, though their significance is doubtful; when $p=1/2$, however, all frequencies are positive and support the theory on which they are based.

If the conclusions suggested by these results are correct, we should expect that when two strands are recovered (exceptional offspring), usually not more than one strand will be a crossover. This is borne out by the results with the X chromosome: from BRIDGES and ANDERSON (1925 table 5) it appears that a crossover chromatid is associated with a non-crossover 82 times out of 97, and with a crossover only 15 times. The tendency is complete at the spindle fibre and decreases for more distal regions because of crossing over between them and the spindle fibre. In the autosomes there is a similar relation between association of chromosomes and distance from the spindle fibre (REDFIELD 1930). As has been pointed out by MULLER (unpublished, cited by WEINSTEIN 1932c), these facts support the view that only two of the chromosomes conjugate while the third remains unmated, although there may be some change of partners.

Similar conclusions concerning conjugation in triploids have been reached by MATHER (1933, 1935); and on cytological grounds by BELLING (1921), MULLER (1922) and DARLINGTON (1932). RHOADES (1933) and KIKKAWA (1934) have presented somewhat different interpretations. The questions raised by these workers cannot be discussed here but will be treated elsewhere.

Frequency of crossing over in diploids and triploids

In comparing the amount of crossing over in diploids and triploids, it is necessary to distinguish between crossing over per strand and crossing over per tetrad or hexad (WEINSTEIN 1932a).

If in any given region crossing over is constant per chromatid, then if chromatids are recovered at random there would be no change in crossing over per chromatid in triploids if sister strands do not cross over, but an increase if they do. For in the latter case one third of the crossings over in a tetrad would be undetectable, whereas in the hexad the proportion would be only $3/15=1/5$. This was pointed out by BRIDGES and ANDERSON (1925), who suggested that thus it might be possible to discover whether or not crossing over occurs between sister strands.

There is however another possibility which they did not consider; namely, that the amount of crossing over might be constant not per strand but per group of chromatids (tetrad or hexad). In this case the amount per chromatid would be decreased, since there would be the same number of crossings over but more strands. The ratio of recognizable crossover strands in triploids to that in diploids would then be $4/15 \div 1/3 = 4/5$ if sister strands cross over, and $1/3 \div 1/2 = 2/3$ if they do not; provided of course that strands are recovered at random. Here again it might be possible to discover whether sister strands cross over.

In BRIDGES and ANDERSON's data for the X chromosome, the amount of crossing over per strand recovered was half as great in triploids as in diploids (except for the leftmost region of the X, which showed an increase). If strands were recovered at random, this would agree better with the supposition that crossing over is the same for hexad as for tetrad and that sister strands do not cross over. But the decrease is too great and suggests that non-crossover strands are recovered more often than on chance. If two strands are recovered and one is usually a non-crossover, the amount of crossing over per recovered strand in triploids would be approximately halved, as it is; hence the figures support the theory that only two strands usually associate in synapsis while the third goes unmated to one pole. We may still conclude that crossing over per hexad is the same as per tetrad; but no conclusion can be drawn directly as to crossing over between sister strands, for the result would be the same whether this occurs or not, since the hexad acts essentially as a tetrad.

At the left end of the X, where there must be a greater tendency for strands to be recovered at random (because of crossing over between this region and the spindle fibre), the ratio might be expected to approach 2/3 if sister strands do not cross over; instead it rises to 2. This increase remains unexplained.

In REDFIELD's data, since only those individuals were selected that did not receive the unmated chromosome, the triploid-diploid ratio per strand might be expected to decrease from 1 at the spindle fibre to about 2/3 for distal regions, where strands are recovered more nearly at random. The latter figure is realized near the ends of the chromosome; and there is a rise toward the center. Near the spindle fibre however the ratio increases to 3 or 4; and this increase is not accounted for.

MULLER (unpublished, see WEINSTEIN 1932c) and RHOADES (1933) have reached similar conclusions, though RHOADES's interpretation of synapsis in triploids differs from that given above.

Higher polyploids

The multiple-strand theory can be applied to higher polyploids; it is

merely necessary to take into account which chromosomes are distinguishable and how they undergo synapsis. This may result in complicated situations; but where (as in some tetraploids) synapsis is in pairs, the treatment is not essentially different from that of diploids.

SUMMARY, WITH SOME CONSIDERATION OF THE MECHANISM OF CROSSING OVER

A mathematical method is described which makes it possible to calculate from the observed frequencies in a crossover experiment (1) the frequency of undetected crossing over including that between sister strands, which cannot be recognized directly, and (2) how the individual chromatids are associated in tetrads. The method can be applied to ordinary diploids, to cases of non-disjunction and attached chromosomes, and to polyploids. The calculated results differ according to the assumptions made as to crossing over at any given level and the mutual relations of crossings over at different levels; and since some of the results are inconsistent with the experimental data or (as in the case of negative frequencies) meaningless, the assumptions on which they rest can be ruled out and our knowledge of the mechanism of crossing over thus becomes more precise.

The experimental results when subjected to this mathematical treatment lead to the following conclusions regarding the mechanism of crossing over:

(1) There is no crossing over between sister chromatids.

(2) At any level only two of the four chromatids may cross over.

(3) Otherwise it is a matter of chance which chromatids cross over at any level.

(4) The chromatids that cross over at one level do not determine which ones cross over at other levels.

(5) This mechanism implies that for inclusive coincidence the true value for strands or tetrads is identical with the observed value regardless of whether sister strands cross over or not; for select coincidence the tetrad value is less than the observed value where the two differ; and for partial coincidence the tetrad and the observed values are in general not identical, though they may be very similar. If recurrence is not random, all types of tetrad coincidence will in general differ from the observed values.

Coincidence for chromatids remains identical with the observed values if sister strands do not cross over.

All these propositions and others are summed up in formula 2 for $p = 1/2$; that is, that the frequency of any class of tetrads is given by

$$X = 2^r[a_0 - a_1 + a_2 - a_3 + \cdots + (-1)^n a_n].$$

(6) In triploids the same mechanism holds; but the evidence indicates

that two of the chromosomes undergo synapsis to the exclusion of the third, over considerable distances, though there is some change of partners; but recurrence is probably random among the strands of the conjugating chromosomes.

Deviations from the mechanism described above lead to results that are inconsistent or incorrect:

(1) Crossing over between sister chromatids would involve negative frequencies of tetrads, would not allow the proportion of homozygosis for any gene to exceed 16 2/3 percent in cases of attached X's and non-disjunction and would result in other discrepancies. Evidence in the same direction is available in the work of EMERSON and BEADLE (1933) and BEADLE and EMERSON (1935) on attached X's; and of L. V. MORGAN (1933) on the ring-shaped X; and in the non-occurrence of sister-strand crossing over in Bar either in its ordinary locus (STURTEVANT 1925) or when translocated to the left end of the chromosome (MULLER and WEINSTEIN 1932 and unpublished data).

(2) Crossing over between more than two chromatids at a given level would diminish or eliminate homozygosis in offspring of attached X's. This conclusion is supported by the non-occurrence of identical crossovers in offspring of attached X's (ANDERSON 1925b) and of triploids (BRIDGES and ANDERSON 1925).

(3) Random occurrence of crossing over is shown most simply in the equality of lateral and diagonal crossing over near the spindle fibre (or a 2:1 ratio if sister strands cross over); and this involves random occurrence in other regions to give the observed results.

(4) Deviations from random recurrence would, if too great, lead to negative frequencies, and would modify the proportions of homozygosis in attached X's from the observed values.

The above results would follow the modification of one condition at a time. Modification of two or more conditions simultaneously might increase discrepancies; for example, the crossing over of all four chromatids at the same level would produce some of the same effects as an increase of digressive crossing over. On the other hand, some modifications would compensate for one another: an increase in digressive crossing over will counteract some effects of increased regressive crossing over, and it may also eliminate negative frequencies produced by crossing over between sister strands.

The conclusions have been stated in terms implying that at the time when crossing over occurs each chromosome is split into two separate strands or rows of genes. This assumption is not required by the mathematical analysis, and it leads to some difficulties which, together with the modifications they suggest, will be briefly considered.

(1) Crossing over between diagonal chromatids might prevent interchange between two others at the same or neighboring levels by keeping them apart; but it is not obvious why a lateral crossing over should have the same effect. This suggests that when crossing over occurs sister chromatids are not completely independent of each other.

(2) It is difficult to see why two completely independent sister chromatids should not cross over with each other, particularly since a sister chromatid might change positions with a homologous one by crossing over with it. The evidence however does not actually show that sister strands do not cross over: such crossings over are not directly detectable since they result in no recombination of characters. Our criterion of their occurrence is really whether crossing over between homologous strands at the same or neighboring levels is prevented, and the evidence shows that it is not. This could be explained if the sister genes are not arranged in two distinct rows but the genes at one level are oriented at random with respect to the genes at other levels; or to put it somewhat differently, there would be one row of double genes instead of two rows of single genes, and there would be no sister strand crossing over because there would be no sister strands (WEINSTEIN 1932c).

It is not necessary however to assume that orientation of genes at one level is random with respect to all other levels; but only that it is random with respect to the next level at which crossing over occurs—a considerable distance, being in the X chromosome of Drosophila some 14 units or about one-fifth of its genetic length. It would be sufficient if within this distance orientation of genes were random on two sides of one interlocus; or if genes did tend to form two strings, but the tendency of two genes to remain in the same string decreased with distance between them and finally disappeared when the distance became long enough for a second crossing over to occur. This might help to explain also why there is crossing over at all.

(3) It is difficult to understand why two chromatids that are sufficiently closely associated to exchange parts are no more likely to associate together at the next crossing over than either is to associate with a third. If at levels sufficiently far apart the orientation of sister genes is random, this difficulty disappears.

Thus all three difficulties suggest that the two chromatids of a chromosome are not entirely separate strands, and two of the difficulties can be explained if the orientation of sister genes at one level is random with respect to the sister genes of the same chromosome at other levels at which crossing over occurs.

The limitation of crossing over at a given level to two chromatids requires however further explanation. If both sister genes are already formed at the time that crossing over occurs, it must for some reason be difficult

or impossible for both to become detached from their neighbors in the chromosome. This might be due to the persistence of a material connection between successive genes, or of some physical force.

It is possible however that both sister genes are not already formed before crossing over takes place; particularly if the new genes are formed not by division of the old ones but by being catalyzed by them. The limitation of crossing over could then be explained if the old genes remain linked at the points of crossing over and only the new genes can become attached to new genes of the other chromosome, more or less as BELLING (1933) postulated. This might be brought about by the formation of the new genes on different sides of the old genes at different levels. There would still however have to be emerging chromatids made up of old and new genes of the same chromosome, to account for progressive crossing over; and this would come about if as the chromatids separate, old and new genes move at random at different levels of crossing over, as has been suggested above.

HISTORICAL NOTE

The part of the present investigation dealing with random recurrence and constant p was carried out in 1928. An abstract published in that year (WEINSTEIN 1928) included a statement of the problem, the general formula numbered 2, the invariance of coincidence under certain conditions, and the applicability of the method to attached X's and polyploids. The conclusion was drawn that association of strands in crossing over can not be entirely a matter of chance; this was based on the application of the formula for various values of p, including 1/3 (free sister-strand crossing over) and 1/2 (no sister-strand crossing over). The crossover frequencies worked out by BELLING (1931) and SAX (1932) correspond to the case $p = 1/2$ if different classes of the same rank are not separated.

The theory was generalized by WEINSTEIN (1930); and the complete theory was presented in a paper and exhibits at the Sixth International Congress of Genetics (WEINSTEIN 1932a, 1932b). The exhibits included the originals of all the figures in the present paper, but the additional data based on table 8 have since been added to figure 4. The work on attached X's and triploids was reported in the same year (WEINSTEIN 1932c).

The derivation of formulas by sets of equations, as given in the present paper, was included in the writer's report before the Congress of Genetics in 1932. Special cases of such sets of equations have since been used by MATHER (1933, 1935) and KIKKAWA (1934).

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THE TRISOMIC DERIVATIVES OF OENOTHERA LAMARCKIANA

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INTRODUCTION

IN DESCRIBING the chromosome configurations in certain trisomic oenotheras, CATCHESIDE (1933) has shown how the origin and characteristic behavior of the trisomic forms can be accounted for by the translocation interpretation of multivalent ring formation.[1] There are, however, so many respects in which the trisomic derivatives of the oenotheras differ from those of other organisms that a fuller discussion of the entire problem is desirable. Since the derivatives of *Oenothera Lamarckiana* have been studied in greater detail than those of other species, the following discussion will be confined almost entirely to the former, though the situations described should be very much the same in other species of the genus in which there are large chromosome rings.

The principal respects in which the trisomics of *Oe. Lamarckiana* differ from similar forms in other genera are: 1, the higher frequencies in which the trisomic forms appear in the progenies of diploids; 2, the larger number of distinct trisomic types produced directly from the diploid; 3, the larger number of "secondary" trisomic types produced in the progenies of the "primaries"; 4, the production of identical types both as "primaries," directly from the diploid, and as "secondaries," in the progenies of other trisomics; 5, the dissimilar breeding behavior observed in different trisomics: whereas certain trisomic derivatives of *Oe. Lamarckiana* always throw the ancestral, diploid type in their progenies, as do the trisomics of most other organisms, other trisomic derivatives of *Oe. Lamarckiana* breed true for the trisomic condition.

By the application of the translocation interpretation to the observed cytological behavior of the diploid and trisomic forms, these peculiarities of the oenothera trisomics may be readily accounted for. The following sections of the present discussion will treat each of these peculiarities separately in their relation to the currently accepted interpretations.

CHROMOSOME DISJUNCTION IN HETEROZYGOUS TRANSLOCATIONS

In forms heterozygous for a reciprocal translocation, the four chromosomes which make up the closed ring may separate in any of three ways

[1] Earlier, CLELAND (1929b) had discussed the relation of non-disjunction of ring-chromosomes to the production of trisomics, but this was not done in relation to the translocation interpretation and consequently certain erroneous conclusions were drawn.

in the first meiotic anaphase. Only one of these types of disjunction gives products carrying the full complement of chromosome ends, and this is the type illustrated in figure 1A in which the chromosomes assume a zigzag orientation on the meiotic spindle so that each chromosome separates from the two adjacent chromosomes which are homologous to it over parts of their lengths. In the other two types of disjunction (figures 1B and 1C), adjacent chromosomes pass to the same pole, and the products in each instance are deficient for one chromosome arm and carry another arm in duplicate.

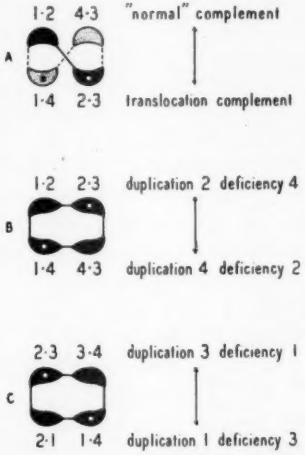

FIGURE 1.—Diagrams of the three types of disjunction possible in a ring of four chromosome The arrows indicate the directions in which the chromosomes separate.

In *Drosophila melanogaster*, gametes carrying a net duplication for one chromosome arm and a deficiency for another are functional provided they meet gametes carrying the complementary product of the same type of disjunction (B or C figure 1). Genetic data indicate that the regular, zigzag disjunction occurs in approximately 60 percent of the instances in one such translocation (DOBZHANSKY and STURTEVANT 1931), and one or the other of the two non-disjunctional types in the remaining instances.

In plants, on the other hand, microspores receiving a deficiency for one chromosome arm and a duplication for another do not develop into functioning pollen. *Zea mays* heterozygous for a reciprocal translocation produces, in most instances, about 50 percent of small, empty pollen grains, and the regular, zigzag disjunction has been observed to occur in about half of the microspore mother-cells and the non-disjunctional types in the other half (BRINK and BURNHAM 1929, BURNHAM 1930, MCCLINTOCK 1930, BRINK and COOPER 1932).

In oenotheras heterozygous for a single reciprocal translocation, the ring chromosomes almost invariably separate in the regular, zigzag manner. Such an oenothera is the hybrid *flavens·stringens* in which CLELAND and OEHLKERS (1930) found but two instances of non-disjunction in a total of 109 sporocytes examined. Consequently there is little or no bad pollen produced by oenotheras with small rings of chromosomes; for example *velans·ʰHookeri*, DAVIS' strain of *Oe. franciscana*, etc. In oenotheras heterozygous for a large number of translocations, that is in oenotheras with large rings of chromosomes, the non-disjunctional types occur more frequently. These types of disjunction will be described for *Oe. Lamarckiana*.

CHROMOSOMAL CONSTITUTION OF OE. LAMARCKIANA

Oenothera Lamarckiana is a heterogametic species in which one haploid set of chromosomes is not identical with the other. Of the seven chromosomes making up each set, only one is common to both; the remaining six chromosomes of one set represent translocations of the six remaining chromosomes of the other set. Hence, in the ordinary diploid form of this species there are thirteen chromosomes of more or less different homologies, only one of which is represented in duplicate.

FIGURE 2.—Diagram of the chromosome configuration at metaphase or early anaphase in *Oe. Lamarckiana*.

Note—In this and succeeding diagrams the following conventions are used: the ring-chromosomes belonging to the *gaudens* complex are distinguished by dots within the chromosomes and by sloping numerals (in the text these chromosomes are represented by Italics, the *velans* ring-chromosomes and the pairing chromosomes by Roman type); whenever the chromosomes form a closed ring, as in this diagram, the ring is represented in side view with the chromosomes in the foreground drawn in solid black and those in the background lightly shaded.

The two haploid sets of chromosomes in *Oe. Lamarckiana* are known respectively as the *velans* and *gaudens* complexes. All the chromosomes making up the *velans* complex have been identified (CLELAND and BLAKESLEE 1930, EMERSON and STURTEVANT 1931, RENNER 1933). The chromosomes of *velans* are designated 1·2, 3·4, 5·8, 6·7, 9·10, 11·12, 13·14, in which each number represents a particular chromosome end. The chromosomes of the *gaudens* complex have not been completely identified. Chromosomes 1·2 and *5·6* are known to be present in this complex, and one of the remaining possibilities for the other chromosomes is *3·9, 4·12 7·11, 8·14, 10·13*, which will be used tentatively in this treatment for which an exact identification is not essential.

The pairing of homologous regions of the chromosomes of the two complexes brings twelve of the chromosomes into a large ring in which the chromosomes of *velans* and *gaudens* must alternate as indicated in the accompanying diagram (figure 2). Then the regular, zigzag disjunction of chromosomes in anaphase must separate the *velans* ring-chromosomes from those of *gaudens* and only the two normal complexes should result.

IRREGULAR DISJUNCTION OF CHROMOSOMES

While, as a rule, adjacent ring-chromosomes pass to opposite poles in meiosis, it is often observed, especially in the case of large rings, that adjacent chromosomes may pass together to the same pole. CLELAND (1929a) examined 358 microsporocytes of *Oe. Lamarckiana* in each of which the ring of twelve chromosomes was intact. Of these 67, or 18.7 percent, had irregularities in the zigzag arrangement of chromosomes.

In a ring made up of an even number of chromosomes such irregularities cannot occur singly, since if two adjacent chromosomes pass to one pole it is impossible for all remaining chromosomes to assume the zigzag orientation. The two sets of irregularities which must occur may have different relative positions in the ring, and the products resulting will differ depending upon the positions of the irregularities.

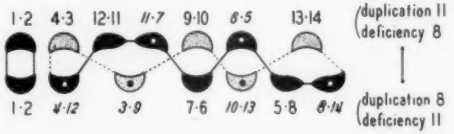

FIGURE 3.—Non-disjunction of ring-chromosomes in *Oe. Lamarckiana* from which seven chromosomes are recovered in each product.

Numerically equal non-disjunction

In those instances in which one set of irregularly disjoining chromosomes passes to one pole and the other set to the other pole (see CLELAND 1929a, figure 23) the disjunction of chromosomes is numerically equal. In this sort of separation, however, the two complexes are not recovered intact, but at each pole there is a mixture of *velans* and *gaudens* chromosomes. At one time (CLELAND 1929b) it was thought that such irregularities might be the basis of genetic crossing over in these forms. Since the establishment of the translocation hypothesis of ring formation, however, it is known that such irregularities in disjunction must give rise to inviable products which will contribute largely to the bad pollen and inviable eggs observed in *Oe. Lamarckiana*. The distribution of homologous segments of chromosomes in disjunctions of this sort is illustrated in figure 3. One daughter cell receives one chromosome arm in duplicate, for example arm 11, but

lacks another arm entirely, arm 8 in this instance, whereas the other daughter nucleus fails to receive the former and has the latter in duplicate. Spores in which there is a deficiency for one chromosome arm and a duplication for another generally fail to produce functioning pollen, as has been well illustrated in the different examples of translocations in Zea (BRINK and BURNHAM 1929, BURNHAM 1930, BRINK and COOPER 1932, etc.).

Numerically unequal disjunction

In other instances (CLELAND 1929a, figures 25, 26, CLELAND and OEHLKERS 1930, figures 1D, 4D, 6H, 9E, 11F, 12E, 12H, etc.), both sets of irregularly disjoining chromosomes pass to the same pole, resulting in an unequal distribution in which six chromosomes pass to one pole and eight to the other. Examples of this sort are illustrated in figures 4 to 6. The daughter cell receiving six chromosomes is deficient for two chromosome arms (11 and 14 figure 6) and should fail to develop either as megaspore or microspore. The cell receiving eight chromosomes, however, has a full

FIGURE 4.—Non-disjunction in which three adjacent ring-chromosomes pass to the same pole, resulting in an 8 to 6 distribution of chromosomes.

complement of chromosome arms and, in addition, two arms in duplicate (ends 11 and 14 in the example illustrated). Eight-chromosome complexes of this sort generally fail to function as pollen but are readily transmitted through the eggs.

CLELAND (1929a and CLELAND and OEHLKERS 1930) has studied the frequency in which such numerically unequal divisions occur in different strains of *Oe. Lamarckiana*. The observed frequency was about 10 percent in the "1912 selfed" strain of SHULL, the *Lamarckiana* of DE VRIES and in the r-*Lamarckiana* of RENNER, but approximately 20 percent in the "1910 selfed" strain of SHULL and in *Oe. Lamarckiana cruciata*.

In the 8-chromosome products of such irregular disjunctions there are mixtures of *velans* and *gaudens* chromosomes; the extent of the mixing depends upon the relative positions in the ring of the two sets of irregularly disjoining chromosomes. In certain instances, such as illustrated by CLELAND and OEHLKERS 1930, figure 12E, three adjacent chromosomes may pass to one pole, in which case the cell receiving eight chromosomes has either a complete set of *velans* chromosomes together with one from *gaudens* (figure 4), or a complete set of *gaudens* chromosomes and one from

velans, depending upon the exact positions of the irregularities. Since there are twelve positions in the ring at which such irregularities may occur, there will be twelve different 8-chromosome complexes (those numbered 1 to 12 in table 1) arising from this particular sort of disjunction.

Following a second type of unequal distribution, in which the two sets of adjacent chromosomes which pass to the same pole are separated by one chromosome which goes to the opposite pole (figure 5), the 8-chromosome products will again have seven ring-chromosomes, but there will be either five *velans* and two *gaudens*, or two *velans* and five *gaudens*. In each of the

FIGURE 5.—Non-disjunction in which the 8-chromosome products have 7 ring chromosomes, including either 5 *velans* and 2 *gaudens* or 2 *velans* and 5 *gaudens*.

twelve different 8-chromosome complexes (13 to 24 in table 1) which arise from this particular type of disjunction, there is no one particular chromosome which can be considered as an extra chromosome; instead, such complexes carry net duplications for two chromosome arms which taken together do not represent any chromosome normally present in *Lamarckiana*. The same is true of the twelve complexes (25 to 36 in table 1) arising in the manner illustrated in figure 6. In such cases there are three regularly disjoining chromosomes lying between the two sets of non-disjoining chromosomes, and the 8-chromosome products have either four *velans*

FIGURE 6.—Non-disjunction with 3 regularly disjoining chromosomes lying between the 2 sets of non-disjoining chromosomes, giving 8-chromosome products with 4 *velans* and 3 *gaudens* ring-chromosomes.

ring-chromosomes and three *gaudens*, or three *velans* and four *gaudens*, again depending upon the exact positions of the irregularities.

There is thus a total of thirty-six complexes with extra chromosomes which can arise directly from *Oe. Lamarckiana* as a result of irregularities in the distribution of the ring chromosomes. All these are composed of mixtures of *velans* and *gaudens* chromosomes in varying degrees. In addition, each represents a net duplication for two chromosome arms, but, as

TABLE 1

The thirty-six different 8-chromosome complexes produced directly from Oe. Lamarckiana by irregular chromosome disjunction.

1	1·2, 3·4, *3·9*, 5·8, 6·7,	9·10, 11·12, 13·14—*velans*+ *3·9*
2	1·2, 3·4, *4·12*, 5·8, 6·7,	9·10, 11·12, 13·14—*velans*+ *4·12*
3	1·2, 3·4, *5·6*, 5·8, 6·7,	9·10, 11·12, 13·14—*velans*+ *5·6*
4	1·2, 3·4, 5·8, 6·7, *7·11*,	9·10, 11·12, 13·14—*velans*+ *7·11*
5	1·2, 3·4, 5·8, 6·7, *8·14*,	9·10, 11·12, 13·14—*velans*+ *8·14*
6	1·2, 3·4, 5·8, 6·7,	9·10, *10·13*, 11·12, 13·14—*velans*+*10·13*
7	1·2, *3·4*, *3·9*, *4·12*, *5·6*,	*7·11*, *8·14*, *10·13*—*gaudens*+ *3·4*
8	1·2, *3·9*, *4·12*, *5·6*, 5·8,	*7·11*, *8·14*, *10·13*—*gaudens*+ *5·8*
9	1·2, *3·9*, *4·12*, *5·6*, 6·7,	*7·11*, *8·14*, *10·13*—*gaudens*+ *6·7*
10	1·2, *3·9*, *4·12*, *5·6*, *7·11*,	*8·14*, 9·10, *10·13*—*gaudens*+ 9·10
11	1·2, *3·9*, *4·12*, *5·6*, *7·11*,	*8·14*, *10·13*, 11·12—*gaudens*+11·12
12	1·2, *3·9*, *4·12*, *5·6*, *7·11*,	*8·14*, *10·13*, 13·14—*gaudens*+13·14
13	1·2, 3·4, *3·9*, 5·8, 6·7,	*10·13*, 11·12, 13·14—duplication 3+13
14	1·2, 3·4, *4·12*, 5·8, 6·7,	*7·11*, 9·10, 13·14—duplication 4+7
15	1·2, 3·4, *5·6*, 5·8, *7·11*,	9·10, 11·12, 13·14—duplication 5+11
16	1·2, 3·4, *5·6*, 6·7, *8·14*,	9·10, 11·12, 13·14—duplication 6+14
17	1·2, 3·4, 5·8, 6·7, *8·14*,	9·10, *10·13*, 11·12—duplication 8+10
18	1·2, *3·9*, *4·12*, 5·8, 6·7,	9·10, 11·12, 13·14—duplication 9+12
19	1·2, 3·4, *3·9*, *5·6*, *7·11*,	*8·14*, *10·13*, 11·12—duplication 3+11
20	1·2, 3·4, *4·12*, *5·6*, *7·11*,	*8·14*, 9·10, *10·13*—duplication 4+10
21	1·2, *3·9*, *4·12*, *5·6*, 5·8,	*7·11*, *10·13*, 13·14—duplication 5+13
22	1·2, *3·9*, *4·12*, *5·6*, 6·7,	*8·14*, *10·13*, 11·12—duplication 6+12
22	1·2, *3·9*, *4·12*, *5·6*, *7·11*,	*8·14*, 9·10, 13·14—duplication 9+14
24	1·2, *3·9*, *4·12*, 5·8, 6·7,	*7·11*, *8·14*, *10·13*—duplication 7+8
25	1·2, 3·4, *3·9*, 5·8, 6·7,	*8·14*, *10·13*, 11·12—duplication 3+8
26	1·2, 3·4, *4·12*, *5·6*, 5·8,	*7·11*, 9·10, 13·14—duplication 4+5
27	1·2, 3·4, *5·6*, 6·7, *8·14*,	9·10, *10·13*, 11·12—duplication 6+10
28	1·2, 3·4, *5·6*, *7·11*, *8·14*,	9·10, 11·12, 13·14—duplication 11+14
29	1·2, *3·9*, *4·12*, 5·8, 6·7,	*7·11*, 9·10, 13·14—duplication 7+9
30	1·2, *3·9*, *4·12*, 5·8, 6·7,	*10·13*, 11·12, 13·14—duplication 12+13
31	1·2, 3·4, *3·9*, *5·6*, 6·7,	*8·14*, *10·13*, 11·12—duplication 3+6
32	1·2, 3·4, *4·12*, *5·6*, *7·11*,	*8·14*, 9·10, 13·14—duplication 4+14
33	1·2, 3·4, *5·6*, *7·11*, *8·14*,	9·10, *10·13*, 11·12—duplication 10+11
34	1·2, *3·9*, *4·12*, *5·6*, 5·8,	*7·11*, 9·10, 13·14—duplication 5+9
35	1·2, *3·9*, *4·12*, 5·8, 6·7,	*7·11*, *10·13*, 13·14—duplication 7+13
36	1·2, *3·9*, *4·12*, 5·8, 6·7,	*8·14*, *10·13*, 11·12—duplication 8+12

can be seen from table 1, no single whole chromosome is represented twice in any one of these 8-chromosome complexes. Each of the first twelve complexes listed has a complete set of the chromosomes of one of the typical complexes (either *velans* or *gaudens*) and one chromosome of the other complex in addition. In these the net duplication corresponds to one of the chromosomes normally present in the diploid form. In the remaining twenty-four extra-chromosome complexes, however, the net duplication is in each instance for two arms which, taken together, do not correspond to any chromosome of either complex of the parent form. Phenotypically the first twelve may be considered as primary "trisomic" complexes and the remaining twenty-four as tertiary in that the net duplications represent possible new translocations. Actually, however, no new translocations are involved.

All trisomic types occurring in the progenies of triploids should be obtainable directly from the diploid as a result of irregular chromosome disjunction. The only possible exception is the trisomic for chromosome 1·2, the pairing chromosome in *Oe. Lamarckiana*. This trisomic is expected in the progeny of triploids, but only following non-disjunction of the pairing chromosome in the progeny of the normal diploid. The complexes (either *velans* or *gaudens*) carrying 1·2 as a duplication together with the thirty-six complexes listed in table 1 are the only 8-chromosome complexes that can be produced by triploids. It might be supposed that 8-chromosome complexes with net duplications other than those already listed might occur, but it is impossible to construct such complexes from the chromosomes present in the triploid form, in which no chromosomes not represented in the diploid form are to be found. For example, there is no 8-chromosome complex listed with the net duplication 3+5. To derive such a complex from a triploid form it would be necessary to recover two chromosomes containing end 3 and two with end 5. These must be 3·4 and 5·8 of *velans* and *3·9* and *5·6* of *gaudens*, since there is no chromosome 3·5 present. Each of the remaining chromosome arms must be represented once and only once to give a viable 8-chromosome complex. Ends 1 and 2 will be represented by chromosome 1·2. End 7 must be represented by chromosome *7·11* of *gaudens*, since 6·7 of *velans* would make the complex a net duplication for end 6 in addition to ends 3 and 5. End 10 must be represented by *10·13* of *gaudens* since 9·10 of *velans* would again produce a further duplication (for end 9). This leaves ends 12 and 14 to be represented and there is no single chromosome in either *velans* or *gaudens* carrying these two arms. Hence it is not possible to have an 8-chromosome complex with a net duplication for arms 3 and 5, unless a new translocation occurs. Similar arguments show that no net duplications other than those listed in table 1, except 1·2, can arise directly from triploid *Lamarckiana*.

TRISOMIC TYPES FROM THE 8-CHROMOSOME COMPLEXES

Were it not for the zygotic lethals carried by certain chromosomes of *velans* and *gaudens*, each of the thirty-six 8-chromosome complexes listed in table 1 should function in two distinct trisomic types, one in conjunction with the normal *velans* complex and the other with *gaudens*. As it is, these characteristic zygotic lethals must greatly reduce the number of trisomic forms obtainable.

The particular trisomic types to be expected cannot be definitely determined until the loci of the zygotic lethals are established. RENNER (1933) has shown that the *velans* zygotic lethal is carried either in chromosome 5·8 or in 6·7, but until the chromosome arm in which it is carried is established we shall not know which chromosome of *gaudens* carries the normal allelomorph of this lethal. The situation is further complicated by our present inability to determine definitely all the chromosomes making up the *gaudens* complex. If *gaudens* actually carries the chromosomes tentatively ascribed to it above, the normal allelomorph of the *velans* lethal may be in any one of chromosomes *5·6*, *7·11*, *8·14*. The *gaudens* zygotic lethal could then be shown to be carried in one of chromosomes *3·9*, *4·12*, *10·13*, or the 11-arm of *7·11*, since none of the *gaudens* chromosomes present in *h deserens* carries the *gaudens* lethal; but here again the chromosome identifications in *h deserens* depend in part upon those tentatively ascribed to *gaudens*. The normal allelomorph of the *gaudens* lethal would then be in one of the *velans* chromosomes 3·4, 9·10, 11·12, 13·14.

Without knowing the exact positions of the lethals in the chromosomes, however, it is still possible to determine the number of different types of trisomics that can be obtained. In table 2 the possible lethal situations for each of the 8-chromosome complexes of table 1 are listed. From this table it can be seen that, regardless of the exact location of the lethals, some complexes will be effectively lethal-free and capable of functioning with both *velans* and *gaudens*. Others will carry one lethal, and not the normal allelomorph of that lethal, and consequently can function with only one *Lamarckiana* complex. Still others may carry both lethals and neither normal allelomorph and consequently will never occur in a viable trisomic type, since such complexes will be inviable with either normal *velans* or normal *gaudens*.

From table 2 it can be seen that complexes 1 to 6, in which there is a full complement of *velans* chromosomes with the extra chromosome from *gaudens*, will each form viable trisomic types with the normal *gaudens* complex regardless of the location of the *gaudens* lethal; and no matter which chromosome arm carries the *velans* lethal, one and only one of these complexes will also be capable of functioning with *velans*. The particular 8-chromosome complex that can function with both *velans* and *gaudens*,

TABLE 2

The distribution of lethals in the various 8-chromosome complexes for all possible positions of the lethals. Symbols: 1=lethal present; c=lethal present but "covered" by presence of normal allemorph; 0=lethal absent.

	velans LETHAL IN				*gaudens* LETHAL IN						
COMPLEX	ARM 5	ARM 6	ARM 7	ARM 8	ARM 3	ARM 4	ARM 9	ARM 10	ARM 11	ARM 12	ARM 13
1	1	1	1	1	c	0	c	0	0	0	0
2	1	1	1	1	0	c	0	0	0	c	0
3	c	c	1	1	0	0	0	0	0	0	0
4	1	1	c	1	0	0	0	0	c	0	0
5	1	1	1	c	0	0	0	0	0	0	0
6	1	1	1	1	0	0	0	c	0	0	c
7	0	0	0	0	c	c	1	1	1	1	1
8	c	0	0	c	1	1	1	1	1	1	1
9	0	c	c	0	1	1	1	1	1	1	1
10	0	0	0	0	1	1	c	c	1	1	1
11	0	0	0	0	1	1	1	1	c	c	1
12	0	0	0	0	1	1	1	1	1	1	c
13	1	1	1	1	c	0	1	1	0	0	c
14	1	1	c	1	0	c	0	0	1	1	0
15	c	0	0	1	0	0	0	0	c	0	0
16	0	c	1	0	0	0	0	0	0	0	0
17	1	1	1	c	0	0	0	c	0	0	1
18	1	1	1	1	1	1	c	0	0	c	0
19	0	0	0	0	c	0	1	1	c	0	1
20	0	0	0	0	0	c	0	c	1	1	1
21	c	0	0	1	1	1	1	1	1	1	c
22	0	c	1	0	1	1	1	1	1	c	1
23	0	0	0	0	1	1	c	0	0	1	0
24	1	1	c	c	1	1	1	1	1	1	1
25	1	1	1	c	c	0	1	1	0	0	1
26	c	0	0	1	0	c	0	0	1	1	0
27	0	c	1	0	0	0	0	c	0	0	1
28	0	0	0	0	0	0	0	0	c	0	0
29	1	1	c	1	1	1	c	0	1	1	0
30	1	1	1	1	1	1	1	1	0	c	c
31	0	c	1	0	c	0	1	1	0	0	1
32	0	0	0	0	0	c	0	0	1	1	0
33	0	0	0	0	0	0	0	c	c	0	1
34	c	0	0	1	1	1	c	0	1	1	0
35	1	1	c	1	1	1	1	1	1	1	c
36	1	1	1	c	1	1	1	1	0	c	1

however, can be definitely determined only when the exact position of the lethal is known. Similarly, complexes 7 to 12 will each form viable zygotes with *velans* and one of them will also be capable of functioning with *gaudens*.

Among complexes 13 to 18, in which two of the ring-chromosomes have been derived from *gaudens* and the remainder from *velans*, there must be one complex which will be lethal with *gaudens*, but there will also be two complexes which will not be lethal with *velans*. Similarly, among complexes 19 to 24, five must be capable of functioning with *velans* and two with *gaudens*. Only four of complexes 25 to 30 can function with *gaudens*, but three must form non-lethal zygotes with *velans*, and similarly in complexes 31 to 36 there will be four which form viable products with *velans* and three with *gaudens*.

Hence, no matter where the characteristic zygotic lethals of *velans* and *gaudens* are located, there must be a total of forty-two different trisomic types resulting from the thirty-six complexes listed in table 1. These, together with the form trisomic for chromosome 1·2, represent all the trisomic types that can arise directly from either the diploid or triploid forms of *Oe. Lamarckiana*.

GENETIC AND CYTOLOGICAL CHARACTERISTICS OF THE DIFFERENT TRISOMICS

The forty-two different trisomics resulting from the thirty-six 8-chromosome complexes of tables 1 and 2 fall into six groups, or classes, each with its own peculiar cytological and genetic potentialities. All members of each group should have identical chromosome configurations, but they may be divided into sub-groups on the basis of the preponderance of *velans* or *gaudens* chromosomes.

Chromosome configurations

Group 1. To the first group of trisomics belong all those that have a complete set of the chromosomes normally present in *Oe. Lamarckiana* with one of the ring-chromosomes present as an extra chromosome. There are twelve possible trisomics of this sort. Each of complexes 1 to 6 (table 1) has a complete set of *velans* chromosomes plus one *gaudens* chromosome, and each will form a viable compound with the normal *gaudens* complex (table 2). Similarly, each of complexes 7 to 12 carries a complete set of *gaudens* chromosomes together with one from *velans*, and each forms a viable product with normal *velans*.

Since the chromosomal constitutions of these twelve trisomics are similar, any one may be used as an example to illustrate the meiotic configurations possible in this group. The trisomic "complex-3·*gaudens*" is

chosen as such an example and the principal configurations are diagrammed in figure 7. The pair 1·2 must always be present, but the remaining chromosomes may be arranged in various ways. If all homologous

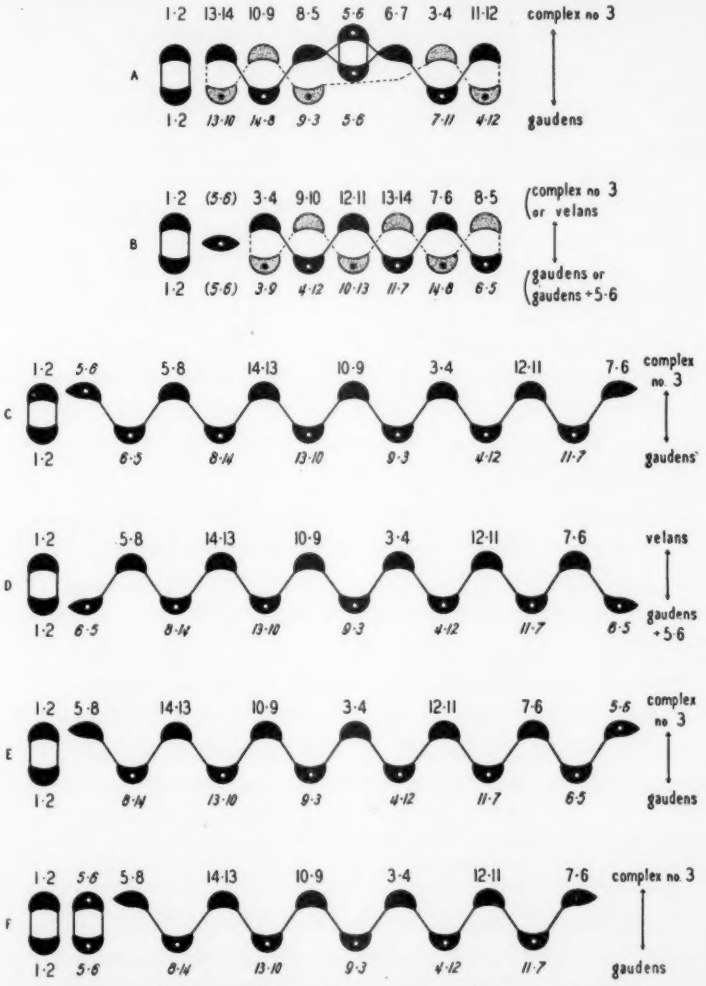

FIGURE 7.—Chromosome configurations in the trisomic "complex-3·*gaudens*."

ends are associated, the two identical chromosomes of *gaudens* will constitute a pair intercalated in a closed ring by triple junctions (figure 7A). All other possible configurations have incomplete association of homol-

ogous ends. The extra chromosome may appear as a univalent with the remaining chromosomes forming a closed ring of twelve (figure 7B); or the extra chromosome may pair with its homolog leaving the remaining chromosomes to form an open chain of eleven (figure 7F); or all thirteen ring-chromosomes may be associated in three different ways to form an open chain of thirteen (figures 7C, D and E).

As an example of trisomics possibly belonging to this group, mut. *stricta* seems to be the best so far studied among the *Lamarckiana* derivatives. HÅKANSSON (1930) has observed a chain of thirteen and one pair in certain sporocytes and a chain of eleven and two pairs in others. *Oe. nutans* mut. *nanella* is a similar trisomic except that it has arisen from a diploid form with a ring of fourteen chromosomes. CATCHESIDE observed many

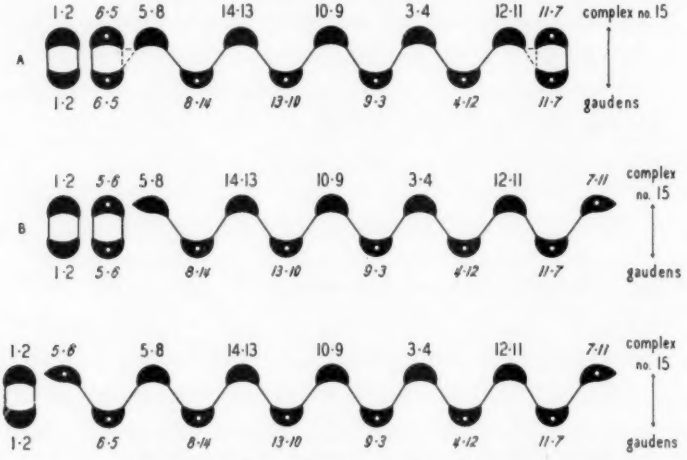

FIGURE 8.—Chromosome configurations in the trisomic "complex-15·*gaudens*."

different configurations: a chain of fifteen, a chain of thirteen and one pair free from the chain, a chain of thirteen with the pair attached at one end by a triple junction (CATCHESIDE, 1933 figs. 2b, 2e and 2f respectively).

Group 2. In the second group there are two chromosomes, in addition to 1·2, carried by both parental complexes, permitting a maximum of three pairs in meiotic prophase. In each of complexes 13 to 18 (table 1) there are two of the *gaudens* ring-chromosomes and five of these complexes (table 2) are viable with normal *gaudens*. Similarly, complexes 19 to 24 have each two of the *velans* ring-chromosomes and five of these complexes are viable with *velans*. The chromosome configurations in each of these ten trisomics will be sometimes a chain of thirteen and one pair (figure 8C), sometimes a chain of eleven and two pairs (8B), and other

times a chain of nine and three pairs (8A). The extra chromosome pairs may be separate from the chain (8B) or either or both may be attached to the ends of the chain by triple junctions as illustrated in figure 8A.

Group 3. The trisomics belonging to the third group will always have two chromosome pairs but may have a maximum of four pairs, with the remaining chromosomes forming a chain in each instance. Figure 9 illustrates the configuration resulting from complete association of homologous ends, from which the other possible configurations may be inferred. The trisomics belonging to this group arise from unions of *gaudens* with the four

FIGURE 9.—Basic configuration in the trisomic "complex-26 · *gaudens*."

complexes of 25 to 30 (table 1) which effectively lack the *gaudens* lethal (table 2) and from unions between *velans* and the four complexes of 31 to 36 which effectively lack the *velans* lethal.

Mutation *curta* may be an example of either group 2 or group 3. The configurations observed in this form were either a chain of nine and three pairs or a chain of eleven and two pairs (HÅKANSSON 1930).

Group 4. In addition to the four complexes of 25 to 30 which effectively lack the *gaudens* lethal, three of these complexes (including at least one which lacks the *gaudens* lethal) either have no *velans* lethal or have it

FIGURE 10.—Basic configuration in the trisomic "complex-26 · *velans*."

covered by the normal allelomorph. These three complexes can thus produce trisomics from unions with normal *velans*. Similarly, three complexes of 31 to 36 can unite with *gaudens* to give viable products. These six trisomics belong to the fourth group in which the chromosome configuration varies between a chain of nine and three pairs, at one extreme, and a chain of five and five pairs at the other extreme (figure 10).

The trisomic form, mut. *oblonga*, studied by CLELAND (1923) may belong to this group. The observed configurations varied between a chain of nine and three pairs, and a chain of three and six pairs; a ring of five chromosomes was sometimes observed. This would seem to be too wide a

range of variability for any one trisomic type. Unquestionably, several different trisomic types have been designated *oblonga;* DE VRIES (1929, p. 130) reports a trisomic, *persicaria*, which occurs in large numbers in the progeny of mut. *scintillans*, but which had previously been overlooked and may have been confused with *oblonga*. CLELAND does not state the origin of his material and it is not certain whether or not he was dealing with a single type.

Group 5. The trisomics belonging to the fifth group arise from the two complexes of 13 to 18 which effectively lack the *velans* lethal when these

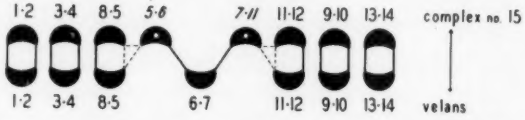

FIGURE 11.—Basic configuration in the trisomic "complex-15·*velans*."

have united with *velans*, and from unions of *gaudens* with the two complexes of 19 to 24 which effectively lack that lethal. These four trisomics have configurations varying between a chain of seven and four pairs and a chain of three and six pairs as extremes (figure 11).

Group 6. The remaining two trisomics should have chromosome configurations varying between a chain of five and five pairs, at one extreme, and seven pairs and a univalent at the other (figure 12). One of these arises from a union of *velans* and that complex of 1 to 6 which carries the normal allelomorph of the *velans* lethal, and the other from a union of *gaudens*

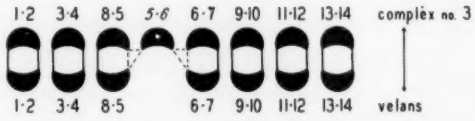

FIGURE 12.—Basic configuration in the trisomic "complex-3·*velans*."

with the complex among 7 to 12 which has the *gaudens* lethal effectively suppressed.

Breeding behavior

Group 1. The type of segregation occurring in the progenies of the various trisomics can be inferred from the chromosome configurations characteristic of each. In the trisomics belonging to the first group, as outlined above, the chromosomes may be oriented in several different ways on the meiotic spindle. Following the diakinesis configuration of a ring of twelve chromosomes, a pair and a univalent (figure 7B), the six *velans* chromo-

somes of the ring together with one member of the pair, 1·2, will pass to one pole, and the six *gaudens* chromosomes of the ring together with the other member of the pair will pass to the opposite pole, whenever the chromosomes separate in the normal, zigzag manner. The extra, univalent chromosome (*5·6* of *gaudens* in the example illustrated) should pass to either pole at random. Hence, following this configuration, four products should be recovered in approximately equal frequencies: *velans;* *velans* plus the extra chromosome; *gaudens* plus the extra chromosome; and *gaudens*.

There are three different arrangements of chromosomes which result in the diakinesis configuration having a chain of thirteen and one pair. Regular chromosome disjunction following two of these arrangements (figures 7C and 7E) yields two products only: *velans* plus the extra chromosome; and *gaudens*. From the other arrangement (figure 7D) the products are: *velans;* and *gaudens* plus the extra chromosome. This configuration can thus produce the same four products as the preceding, but they are not expected to occur in equal frequencies. The exact frequencies cannot be predicted, but the extra chromosome would be expected to accompany *velans* more often than not in the example illustrated.

In all instances in which the extra chromosome has paired with its homolog (as in figures 7A and 7F), the extra chromosome must invariably accompany one particular complex, and this must be the *velans* complex in all trisomics of this group in which the extra chromosome came originally from *gaudens* (as in example, figure 7); whereas in those trisomics in which the extra chromosome originaly came from *velans* it must regularly accompany the *gaudens* complex. Hence, in any one particular trisomic, the four types of gametes produced should not occur in equal frequencies. In trisomics in which a *gaudens* chromosome is present as the extra chromosome, the products occurring in the higher frequency will be: *velans* plus the extra chromosome; and *gaudens*. In trisomics in which a *velans* chromosome is the extra chromosome, the more frequent products will be: *velans;* and *gaudens* plus the extra chromosome.

The inbred progenies of all trisomics of this group should contain typical diploid *Lamarckiana*, from a union of *velans* with *gaudens*, as well as the parental trisomic type, which can arise either from *velans* plus the extra chromosome when fertilized by *gaudens*, or from *gaudens* plus the extra chromosome fertilized by *velans*. These trisomics which regularly throw the diploid form have been called "dimorphic" mutations by DE VRIES. The following examples will serve to illustrate this type of segregation:

cana selfed: 49 per cent *Lamarckiana*, 35 percent *cana*, 16 percent mutations; n = 180.
pulla selfed: 80 percent *Lamarckiana*, 15 percent *pulla*, 5 percent mutations; n = 661.
liquida selfed: 76 percent *Lamarckiana*, 16 percent *liquida*, 8 percent mutations.; n = 507.
cucumbis selfed: 89 percent *Lamarckiana*, 11 percent *cucumbis;* n = 420

The data quoted are all from DE VRIES, the first three from the 1925 paper, the last from the 1929 paper.

The product formed when a *velans* gamete fertilizes a gamete carrying *velans* plus the extra chromosome will be lethal except in the one trisomic in which the extra chromosome is that one from *gaudens* which carries the normal allelomorph of the *velans* lethal. In that particular trisomic, one of the two trisomics belonging to group six should appear in the inbred progeny in a relatively high frequency. If the trisomic in figure 7 is one of this sort, the trisomic in figure 12 should appear in its progeny. It is possible that the so-called accessory form, *oblonga*, appears in the inbred progeny of *scintillans* in this manner. DE VRIES (1913) reported the following segregation in inbred *scintillans:* 66 percent *Lamarckiana*, 21 percent *scintillans*, 12 percent *oblonga*, 1 percent mutations; n 1259. Similarly, the product formed when a *gaudens* gamete meets *gaudens* plus the extra chromosome will be inviable except in the one trisomic in which the extra chromosome is that one from *velans* which carries the normal allelomorph of the *gaudens* lethal.

The fact that the extra chromosome accompanies one complex more often than the other can be determined only in outcrosses. When the trisomic has been used as the female parent, the frequencies in which the extra chromosome appears in the *velans* and *gaudens* twins gives a direct measure of this preference. The following data from DE VRIES (1913) may be taken as an illustration:

OUTCROSS	DIPLOIDS WITH		TRISOMICS WITH		TOTAL
	VELANS PERCENT	GAUDENS PERCENT	VELANS PERCENT	GAUDENS PERCENT	
lata×*Hookeri*	43	39	17	1	1004
lata×*Cockerelli*	48	21	31	0	1389
lata×*chicaginensis*	55	21	23	1	1093

From these data it can be seen that the extra chromosome of *lata* goes most often with *velans*. In the haploid eggs of a trisomic of this sort, *gaudens* should be more frequent than *velans*, were it not that in the eggs of *Lamarckiana* itself, *velans* is much more frequent than *gaudens*. In outcrosses of *Lamarckiana* to the same pollen parents, DE VRIES (1916) obtained 82 percent *velans* twins and 18 percent *gaudens* twins. In *Oe. Lamarckiana* the frequencies of *velans* and *gaudens* megaspores must be equal, yet most of the functioning eggs in these hybrids of DE VRIES are *velans*, indicating that the *velans* members of the megaspore tetrads develop into embryo sacs more often than the *gaudens* members (RENNER 1921). In the trisomic *lata*, there should be an excess of *gaudens* mega-

spores produced, but if the *velans* megaspores are more likely to develop into embryo sacs, the functioning eggs carrying *velans* might still be in excess. The ratio of functioning eggs in *Lamarckiana* was 4.6 *velans* to 1 *gaudens*, and in *lata* 1.9 to 1. The relatively higher proportion of *gaudens* eggs in *lata* must indicate that *gaudens* megaspores are produced in excess of *velans*.

There should be the same difference in the frequencies of *velans* and *gaudens* in the microspores, and, since the 8-chromosome complexes are inviable in the pollen, the frequencies in which the two normal complexes are produced should be directly determinable from outcrosses in which the trisomics are used as pollen parents. Unfortunately, different outcrosses of *Lamarckiana* (DE VRIES 1913) show as great divergences in the *velans-gaudens* ratio as do outcrosses of the different trisomics to the same female parents. Hence it is unwise to attempt to distinguish between the sub-groups from such data alone.

Trisomic for chromosome 1·2. The trisomic carrying a member of the paired chromosome, 1·2, of *Oe. Lamarckiana* as the extra chromosome should resemble the members of group 1 in its breeding behavior. Since the extra chromosome may accompany either *velans* or *gaudens*, this trisomic should also throw diploid *Lamarckiana* in its inbred progeny. However, there should be no preference in the distribution of the extra chromosome, and *velans* and *gaudens* twins should appear in the same frequencies as in diploid *Lamarckiana*. This form can be easily recognized by its chromosome configuration which should be a ring of twelve and a trivalent group.

Groups 2 to 6. The trisomics belonging to the remaining five groups can be distinguished from those just described in that following self pollination they should breed true for the trisomic condition and never segregate diploid *Lamarckiana*. These trisomics are classed as "sesquiplex" mutations by DE VRIES.

Each trisomic belonging to groups 2 to 6 is made up of one normal *Lamarckiana* complex, either *velans* or *gaudens*, and an 8-chromosome complex in which there is a mixture of *velans* and *gaudens* chromosomes. As can be seen from figures 8 to 12 only the two parental complexes can result from regular chromosome disjunction following any configuration that the diakinesis chromosomes may assume. In the examples in figures 8 and 9, a complete set of *gaudens* chromosomes is always recovered at one pole and the eight chromosomes recovered at the other pole are in part *velans* and in part *gaudens;* in the examples in figures 10 to 12, the recovered products are *velans* and a mixed, 8-chromosome complex. Since the two parental complexes alone are recovered, these forms must breed true (homozygous *velans* and homozygous *gaudens* are lethal as in the

diploid). *Oblonga*, *albida*, *candicans*, etc., are examples of true-breeding trisomics.

Outcrosses in which these trisomics are used as the female parents segregate into two types: a trisomic arising from the 8-chromosome complex, and either the *velans* or the *gaudens* "twin" characteristic of similar *Lamarckiana* outcrosses. The following examples from DE VRIES (1913 and 1923, respectively) illustrate this type of segregation:

	trisomics	*velans*-twin	*gaudens*-twin
oblonga×*chicaginensis*	19%	81%	0
delata×*chicaginensis*	46%	0	54%

Since the extra-chromosome complexes are not transmitted through the pollen, the functioning pollen of these trisomics is either all *velans* or all *gaudens*, depending upon which normal complex is present (DE VRIES 1913 and 1923).

Irregular disjunction in trisomics

The nature of the progenies of inbred trisomics as discussed above deals only with the products of regular disjunction. Irregular distributions of chromosomes in the first meiotic anaphase should occur in trisomic forms as well as in the normal diploid. In the trisomics, however, there is not ordinarily a closed ring made up of an even number of chromosomes, but an open chain in which there is an odd number of chromosomes. Hence it is possible for two adjacent chromosomes to pass to one pole with all remaining chromosomes separating in the regular, zigzag manner, as illustrated in figure 13B, with no necessity for a second, compensating irregularity such as is required in a closed ring of chromosomes.

Whenever there is one set of irregularly disjoining chromosomes in the chain of a trisomic, the products must be one 8-chromosome complex and one 7-chromosome complex (figure 13B). The complex receiving eight chromosomes will have a complete set of chromosome ends and in addition will have two ends in duplicate. The net duplication in this 8-chromosome product, however, will not ordinarily be the same as that in the parental 8-chromosome complex, but will be identical with some other 8-chromosome complex produced by *Lamarckiana* (table 1). There will be a different mixture of *velans* and *gaudens* chromosomes in the 8-chromosome products following irregular disjunction (compare figures 13A and 13B). On the other hand, the product receiving seven chromosomes, following irregular disjunction, will have a mixture of *velans* and *gaudens* chromosomes in which there will be a net deficiency for one chromosome arm (13 in the diagram) and duplication for another (arm 6 in the example illustrated). The seven chromosome products of irregular disjunctions of this sort must consequently be inviable.

Since irregular chromosome disjunction in trisomics results in 8-chromosome complexes different from the parental 8-chromosome complexes, new trisomic forms should be expected in the inbred progenies of practically all trisomics. These "new" trisomics, however, will be identical with certain of those produced directly from *Oe. Lamarckiana*, and if the chromosome constitutions of the trisomics were definitely established, it would be possible to predict just which trisomics should appear in the progeny of any particular one.

The number of trisomic types appearing in the progeny of any particular trisomic depends upon the number of different positions in the open chain at which irregularities in disjunction may occur. Consequently trisomics belonging to the different groups (pp. 210–214) should differ in the number of trisomic types produced.

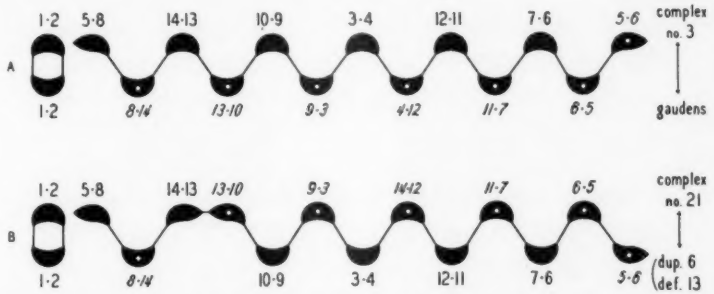

FIGURE 13.—Regular and irregular chromosome disjunction in the trisomic "complex-3 *gaudens*." Regular disjunction (A) yields the two parental complexes; irregular disjunction (B) results in a different 8-chromosome complex and an inviable 7-chromosome complex.

Each trisomic belonging to the first group should have eleven different trisomic types appearing in its progeny as a result of irregular distributions in the open chain of chromosomes. The two members of the group which have the normal allelomorphs of both zygotic lethals in the 8-chromosome complexes, however, should each produce seventeen different trisomic types. These are the numbers of trisomic types that should be produced in fairly high frequencies, but each of the trisomics of this group may occasionally have a ring of twelve chromosomes identical to the ring in *Lamarckiana* (see figure 7B), hence any of the trisomics produced directly from the diploid form may also occur in the progeny of each of the trisomics belonging to group 1, but many of these would be expected in very low frequencies.

The trisomics belonging to the remaining five groups always have open chains and never closed rings. The number of different trisomic types expected in the progenies of these groups are four for each member of

group 2, three for group 3, two for group 4, and one for group 5. The two members belonging to group six should breed true and never throw other trisomic types. Trisomics belonging to the first group should give rise to "secondary" trisomics belonging to all six groups, those belonging to the second group can given rise to "secondary" types belonging to groups 3, 4, 5 and 6, trisomics of the third group to 4, 5 and 6, and so on.

The frequencies in which the different trisomics appear in the progeny of any particular trisomic should be higher than in the progeny of diploid *Lamarckiana*. In the diploid form, about half of the irregular distributions should result in products having each seven chromosomes (see p. 203), whereas any irregularity in the open chain of the trisomic must result in one 8-chromosome product. The published data seem to support this expectation.

DISCUSSION

In the foregoing account an attempt has been made to show how an application of the translocation interpretation to observed cytological behavior can account for the genetic and cytological characteristics of the trisomic derivatives of *Oe. Lamarckiana*. Wherever possible, specific examples from the literature have been quoted to illustrate the different points. There remain certain general and specific attributes of the trisomics which need further consideration.

Frequency of appearance of trisomics. Forms which have since been shown to be trisomics made up 1.2 percent of the progeny (which totaled 53,500) in DE VRIES' original inbred line of *Oe. Lamarckiana* (DE VRIES 1906). It has been shown above that trisomics are expected in the progeny of this species following certain types of irregularities in the zigzag arrangement of chromosomes in first meiotic anaphase. CLELAND (1929a) and CLELAND and OEHLKERS (1930) have shown that in 10 to 20 percent of the pollen mother-cells the chromosomes are distributed eight to one pole and six to the other. If such irregularities are correspondingly frequent in the megaspore mother-cells, 8-chromosome complexes should function as eggs sufficiently often to account for the frequency of trisomics observed by DE VRIES.

Number of different trisomics produced. A rather casual review of the literature shows that probably more than thirty distinct trisomics have been obtained from *Oe. Lamarckiana*. In the preceding analysis it was shown that forty-two or forty-three distinct types were expected. The remarkable thing is that so many have been recorded since many of them are nearly identical in external appearance (see DE VRIES and GATES 1928, DE VRIES 1929). A detailed cytological and genetic study would probably show that more than one type has been included under a particular name (see discussion of *oblonga* and *pulla* below).

Classification of trisomics. DE VRIES and his students have proposed two

classifications for the trisomic derivatives of *Oe. Lamarckiana*. One of these classifications (DE VRIES and BOEDIJN 1923, DULFER 1926) is based on the assumption that, since the haploid number of chromosomes is seven, there should be seven "primary" trisomics in this species. The other trisomics are called "secondaries" because they have occurred principally in the progeny of the primaries, from which they are considered to differ by relatively few genes, as a possible result of crossing over (DE VRIES 1929). The seven groups are:

1. *lata* group: *semi-lata, albida, flava, delata, subovata, sublinearis, latifolia, synedra, planaria;*
2. *scintillans* group: *oblonga, aurita, auricula, nitens, distans, linearis, persicaria,* and some others;
3. *cana* group: *opaca, candicans, tardescens;*
4. *liquida* group: *cucumbis, lingua, plana;*
5. *spathulata* group: *hamata,* and perhaps others;
6. *pallescens* group: *lactuca;*
7. *pulla* group.

Though this classification is based upon a false interpretation, there must be other reasons why certain of the trisomics are sufficiently similar in phenotypic appearance to warrant grouping them together. Different trisomics will be *homozygous* for the same chromosome of *velans* or of *gaudens*, or for more than one chromosome, and each would be expected to have the characteristics determined by those particular chromosomes. Hence it is not surprising that many different trisomics have many phenotypic characteristics in common.

The other classification advanced by DE VRIES is based entirely on the breeding behavior. Two main groups are recognized, the dimorphic mutations which continually throw diploid *Lamarckiana* in their progenies, and the sesquiplex mutations which breed true. The latter are subdivided into a group having *velans* pollen and one having *gaudens* pollen. The dimorphic trisomics could be subdivided on the basis of the origin of the extra chromosome: if it comes from *velans*, most of the pollen and haploid eggs will be *gaudens*, and if the extra chromosome is from *gaudens*, the majority of the haploid gametes will be *velans*. As shown above (p. 214) there should be twelve different "dimorphic" trisomics, or thirteen if the trisomic for chromosome 1·2 is included. The trisomics ascribed to this group are: *pulla, cana, ablata* and *cucumbis*, each with the extra chromosome from *velans; lata, scintillans* and *lingua*, each with the extra chromosome from *gaudens;* and *liquida, pallescens, spathulata, lactuca, hamata* and *superflua*, for which there are not enough data to indicate the origin of the extra chromosome. The data on which this grouping is made are from DE VRIES (1913, 1916, 1925, 1929) and BOEDIJN (1925).

The true breeding "sesquiplex" trisomics which carry a normal *velans* complex are: *albida*, *oblonga*, *candicans*, *auricula*, *aurita*, *persicaria*, *opaca*, *cinerea*, *tardescens* and *planifolia* (DE VRIES 1913, 1923, 1929). Those having a normal *gaudens* complex are: *nitens*, *distans*, *flava*, *delata* and *diluta* (DE VRIES 1923, 1924). There are many other sesquiplex trisomics for which there are insufficient data to indicate their constitutions.

Trisomics involving new translocations. The discussion in the earlier parts of this paper has been confined to trisomics made up entirely of chromosomes normally present in diploid *Oe. Lamarckiana*. There are certain trisomic derivatives of *Lamarckiana*, however, which correspond to the tertiary trisomics of Datura (BELLING 1927) in that certain of the *Lamarckiana* chromosomes have been altered by translocation. The *pulla* studied cytologically by HÅKANSSON (1928) was found to have a ring of six chromosomes, three pairs and a trivalent group. The small ring of chromosomes must have arisen by a new translocation such as is responsible for the appearance of the so-called half-mutants (DARLINGTON, 1931). HÅKANSSON's *pulla*, however, must be different from the form studied genetically by BOEDIJN (1925) since the former could not segregate typical *Lamarckiana*.

The *oblonga* in which CLELAND (1923) observe a closed ring of five chromosomes must again represent a new translocation, in this instance involving the "extra" chromosome, as in the tertiaries of BELLING. Such trisomics as *nitens* and its derivatives, *diluta* and *distans*, which originated from the cross *Lamarckiana*×*blandina*, must have chromosomes with different arrangements of homologous ends from that occurring in *Lamarckiana*, but the translocations in these instances took place in the formation of the *blandina* complex.

Data inconsistent with the interpretation. It was suggested earlier that the "accessory" trisomic *oblonga* which is produced in large numbers by the "dimorphic" trisomic *scintillans*, has two complete sets of *velans* chromosomes and only the extra chromosome from *gaudens*. Designating the extra chromosome as "g," the four types of egg cells possible in *scintillans* are *velans*, *gaudens*, *velans*+g and *gaudens*+g; and the functioning pollen is *velans* and *gaudens*. Then, following self pollination, *velans*×*gaudens* and *gaudens*×*velans* produce typical *Lamarckiana; velans*+g×*gaudens* and *gaudens*+g×*velans* reproduce *scintillans; velans*+g×*velans* results in *oblonga;* and the other possible combinations are lethal.

Data summarised from DE VRIES (1913, 1925, 1929) show about 19 percent *scintillans* and 11 percent *oblonga* in inbred cultures of *scintillans* (n = 2466, which includes data from the cross *scintillans*×*nanella*). In outcrosses to forms with *velans* pollen only there should be fewer *scintillans* since this type will result from only one type of egg, *gaudens*+g, which is relatively infrequent (see p. 215) and there should be more *oblonga*, since all eggs of the constitution *velans*+g should produce this form. In the

cross *scintillans*×*oblonga* (DE VRIES 1913) there were 1 percent *scintillans* and 18 percent *oblonga* (n=63); and in the cross *scintillans*×^{b}*albicans* ·*velans* (DE VRIES 1929) there were 8 percent *scintillans*, 10 percent *oblonga* and 9 percent *persicaria* (n=650). In both crosses the pollen was entirely *velans* and the data support the interpretation. In crosses to *gaudens* (or *rubens*) pollen, on the other hand, no *oblonga* should be produced, since that form arises only when a *velans*+g egg is fertilized by *velans*. The only eggs functioning in this cross are *velans* and *velans*+g, of which the latter should be more frequent (see p. 215). In the cross *scintillans*×*biennis* (all pollen *rubens*), there were 59 percent *scintillans* and 2 percent *oblonga* in a total of 300 plants (DE VRIES 1913). The high frequency of *scintillans* in this cross agrees with the interpretation, but the appearance of a few *oblonga* individuals cannot be accounted for.

Mutation *oblonga* should produce two types of eggs, *velans* and *velans*+g. In outcrosses to *Oe. Lamarckiana* as the pollen parent, *Lamarckiana*, *oblonga* and *scintillans* should be produced, the last named from *velans*+g ×*gaudens*, but apparently *scintillans* does not occur in such crosses. In a total of 244 individuals, DE VRIES (1913) reported 91 percent *Lamarckiana*, 7 percent *oblonga* and only 2 percent of "mutations" which were not specifically mentioned. In outcrosses to *Oe. biennis* in which all the pollen is *rubens* (=*gaudens*), all trisomics produced should be *scintillans* and never *oblonga*. In one such cross (n=63) there were no trisomics, and in another (n=87) 40 percent of the plants were recorded as *oblonga* and another 25 percent as *oblonga*-dwarfs. These results cannot be accounted for by the interpretation outlined above.

For most of the other trisomics, outcross data are too meagre to be used in testing the interpretations placed upon them.

CONCLUSIONS

All the more general genetic and cytological features of the trisomic derivatives of *Oe. Lamarckiana* are made more understandable when considered in relation to the translocation hypothesis. At the present time, however, it is impossible to account definitely for certain specific features of individual trisomics. Detailed cytological studies of particular trisomics and their hybrid-trisomic progenies could doubtless resolve the remaining problems. It should also be possible to determine the homologies of all *gaudens* chromosomes from cytological studies of outcrosses of the various trisomics to the standard "tester" races.

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THE DIFFERENTIATION OF EYE PIGMENTS IN DROSOPHILA AS STUDIED BY TRANSPLANTATION

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INTRODUCTION

PROMINENT among the problems confronting present day geneticists are those concerning the nature of the action of specific genes—when, where and by what mechanisms are they active in developmental processes? Despite the recognized importance of such questions as these, relatively little has been done toward answering them, a situation not at all surprising considering the difficulty of getting at these problems experimentally. Even so, promising beginnings are being made; from the gene end by the methods of genetics, and from the character end by biochemical methods. Probably the one factor which has played the most significant role in retarding progress in this field is the fact that relatively little is known from a developmental point of view about those organisms that have been studied most thoroughly from the genetic point of view, and, on the other hand, little is known genetically in those organisms that have been most studied from the developmental point of view. One of the two obvious (and alternative) ways of overcoming this difficulty would be to study development in a genetically well known organism. Drosophila, with its numerous mutant types, offers a favorable opportunity for a study of this kind. Several facts have led us to begin such a study on the differentiation of eye color pigments. Many eye color mutants are known, pigments have many advantages for chemical studies, and interactions between tissues of different genetic constitutions with respect to eye pigmentation are already known from studies of mosaics.

In this paper we shall present the detailed results of preliminary investigations (EPHRUSSI and BEADLE 1935a, 1935b, 1935c; BEADLE and EPHRUSSI 1935a, 1935b) which we hope will serve to point out the lines along which further studies will be profitable.

MATERIAL AND METHODS

The technique used in making transplantations in Drosophila has been described elsewhere (EPHRUSSI and BEADLE 1936). In brief, the desired organ or imaginal disc, removed from one larva, the donor, is drawn into

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a micro-pipette and injected into the body cavity of the host. As a rule, operations were made on larvae cultured at 25°C for three days after hatching from the eggs. At this time they are ordinarily about ready to pupate. Some of the stocks developed at slower rates than others, and larvae from these were sometimes used on the fourth day after hatching. In most cases the host larvae pupated within 24 hours after the operation. It is clear, from the above, that the stage of development at the time of operations was not controlled in a very precise way. However, since repetition of experiments at different times and, in some cases, with quite different stocks have given consistent results, we can be reasonably sure that the small differences in stage of development which may have existed between host and implant have not played any significant part.

The reasons for the choice of that stage of development reached shortly before puparium formation as "standard" for the studies reported here are largely those of convenience. At this time the optic discs are of a convenient size for transplantation, injections are readily made, and the host larvae require no more food.

As will be discussed below, implanted optic discs develop in a manner somewhat different from that characteristic of the same disc in its normal position. Because of this, it is not always desirable to compare the pigmentation of an implanted eye with that of a normal one. By dissecting the two eyes, normal and implanted, and observing fragments of the pigmented tissue, one can usually make a good comparison. However, to avoid all difficulty, which becomes important where slight differences are involved, we have practically always made comparisons only between implanted eyes. Thus, a vermilion eye disc implanted in a claret host gives rise to an eye with vermilion pigmentation. This conclusion is reached by comparing the implanted eye with an implanted eye known to be vermilion, obtained by implanting vermilion discs in vermilion larvae. Further comparisons with wild type and with claret control implants enable one to say definitely that the eye in question is vermilion, not wild type and not claret.

List of mutants

A list of the eye color mutants used in the studies reported in this paper is given together with their standard symbols. These mutant types and the genes which differentiate them from wild type will be referred to by symbol only. Other mutant genes were also carried by certain of the stocks used. These are indicated in the tables by symbol only since they presumably have no bearing on the results. These symbols are used generally in Drosophila work; their significance can be found in MORGAN, BRIDGES and STURTEVANT (1925).

bo —bordeaux
bw —brown
ca —claret
car —carnation
cd —cardinal
cl —clot
cm —carmine
cn —cinnabar
g^2 —garnet-2
Hn^r—Henna-recessive
lt —light
ma —maroon
p^p —peach
pd —purpleoid
pn —prune
pr —purple
ras —raspberry
rb —ruby
se —sepia
sed—sepiaoid
sf^2 —safranin-2
st —scarlet
v —vermilion
w —white
w^a —apricot
w^e —eosin

DEVELOPMENT OF IMPLANTED EYES

When an eye transplant is made, the eye disc is injected into the body cavity of the host larva. The implanted disc continues development in the body cavity, and at maturity of the host usually comes to lie in the abdominal cavity. Occasionally, it may lie in the thorax but such cases are exceptional. The location of the implanted eye in the adult fly seems to be determined by purely mechanical factors; it is pushed into that part of the body cavity of the developing individual where the normal organs are least crowded. Usually injections are made toward the posterior end of the larva, but they have also been made near the anterior end, and this seems to have no effect on the final position of the eye. The implanted eye may lie just under the body wall of the adult fly where it is readily visible in the living fly, or it may lie deeply imbedded, in which case it may not be visible without dissection or clearing.

Very often the implanted eye becomes attached to other organs during its development. In females, it is often attached to one of the ovaries. This appears to be brought about mainly by the growth of tracheal tubes. In males the implanted eye may be attached to a testis. Males with an implanted eye sometimes have one testis which retains the ellipsoid shape which is characteristic of a testis at a much earlier stage of development. Such "inhibited" testes may have their sheaths normally pigmented but whether they contain viable spermatozoa is not known.

An implanted eye, which has developed within the body cavity of the host, is inverted as compared with an eye in its normal position. The normal eye has the shape of the head of a mushroom, the outer surface of the eye being represented by the top or convex surface of the mushroom head. An implanted eye disc is detached from its optic ganglion and, after development, its curvature is reversed in such a way that the facets are on the inside and the basement membrane on the outer convex surface. In other respects implanted eyes appear to be perfectly developed and differentiated; particularly, there seems to be no difference in the pigmentation of an implanted and a normal eye.

The optic and antennal imaginal discs in the larval stage are attached to each other. In removing an optic disc for transplantation, the antennal disc is usually left attached and implanted with the optic disc. This is not necessary but is done in routine procedure because it facilitates handling the discs and in most experiments does no harm. In special experiments where it may be desirable to do so, it is easy to remove the antennal disc and implant the optic disc alone. If the antennal disc is not removed and is not injured during dissection, it develops with the implanted eye and gives rise to an antenna, complete with an arista, attached to the eye by the chitinous head parts mentioned below. In most instances antennae developing with implanted eyes are normally everted.

The optic disc gives rise also to certain head parts when it is implanted, and presumably also in its development in the normal position. The exact extent of these head parts which arise from the optic disc has not been determined but they completely surround what would normally be the periphery of the eye and have normally developed bristles. As the developed implanted eye is inverted, these chitinous head parts form a kind of rim around the concave facet-side of the eye with the bristles on the inside.

In very exceptional cases an implanted eye disc may give rise to an external eye. This has happened only four times in about 1200 cases. In one of these, the eye was nearly normal, the facets were on the exterior convex surface, and there was a normally developed antenna attached to the eye by chitinous head parts. In all four cases the supplementary eye was attached to the abdominal wall of the adult fly, presumably at the point of injection. These cases are unusual and probably arise when the optic and antennal discs "plug," in a special way, the hole through which the pipette was inserted.

EXPERIMENTAL RESULTS

Because of the rather complex interrelations of the different types of data to be presented in this paper, they cannot be discussed efficiently until all the data have been presented.

In the following tables the various sex combinations of implant and host are given. In only one case, which will be specifically mentioned, does the sex of either the donor or the host appear to influence the result.

Mutant eye discs in wild type hosts

As a beginning in the study of the differentiation of eye pigment of implanted eyes, it is desirable to know how many eye color mutants are autonomous in their pigment development when implanted in wild type hosts. For the late larval stage, with which we are chiefly concerned in

TABLE 1

Data on the differentiation of mutant eye implants in wild type hosts. Eye color mutant symbols are distinguished from symbols of incidental mutants present in the stocks by being printed in italics. In this and following tables, under the heading "number of individuals," are given the four sex combinations and the total in the following order: female in female, male in female, female in male, male in male, and total.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
bo	+	3, 1, 1, 1; 6	?	*pd*	+	2, 0, 2,* 2; 6	*pd*
bw	+	4, 2, 2, 0; 8	*bw*	y *pn*	+	1, 1, 4, 0; 6	*pn*
ca	+	7, 0, 5, 2; 14	*ca*	b *pr*	+	4, 0, 1, 0, 5	*pr*
car	+	5, 0, 6, 0; 11	*car*	sc *ras*	+	2,‡ 0, 0, 0; 2	*ras*
cd	+	4, 0, 0, 0; 4	*cd*	*rb* cv	+	2, 0, 3, 0; 5	*rb*
cl	+	2, 2, 0, 1; 5	*cl*	*se* wo	+	5, 6, 5, 4; 20	*se*
cm	+	1, 0, 2, 1; 4	*cm*	sr *sed*	+	2, 2, 0, 6; 10	*sed*
cn	+	5, 3, 2, 0; 10	+	tk sf^2 abb	+	0, 2, 1, 1; 4	sf^2
cn	+/*v*	2, 0, 2, 0; 4	+	*st*	+	3, 0, 0, 2; 5	*st*
g^2	+	1, 1, 0, 0; 2	g^2	*v*	+	11, 6, 8, 5; 30	+
jv Hn^r h	+	0, 0, 1, 0; 1	Hn^r	*v*	+/*v*	1, 0, 0, 0; 1	+
lt c	+	0, 1, 2, 1; 4	*lt*	*w*	+	1, 1, 1, 0; 3	*w*
ma	+	2, 2, 2, 2; 8	*ma*	w^a	+	0, 0, 3, 0; 3	w^a
p^p	+	2, 0, 1, 1; 4	p^p				

* One fly in this class had an implanted eye with wild type pigmentation—presumably because of a mistake in the selection of the donor.

‡ One host in this class dissected as mature pupa.

TABLE 2

Data on the differentiation of wild type eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
+	*bo*	0, 0, 0, 1; 1	?	+	p^p	3,* 2,* 0, 0; 5	+
+	*bw*	2, 1, 1, 0; 4	+	+	*pd*	2, 0, 6, 0; 8	+
+	*ca*	2, 2, 2, 0;		+	y *pn*	2, 4, 0, 1; 7	+
		6,* 1*; 13	*ca*				
+	*car*	1, 0, 1,‡ 0; 2	+	+	b *pr*	3, 1, 4, 1; 9	+
+	*cd*	4, 1, 0, 0; 5	+	+	sc *ras*	1, 1, 1, 1; 4	+
+	*cl*	2, 2, 5, 1; 10	+	+	*rb* cv	1,‡ 0, 2, 1; 4	+
+	*cm*	2, 1, 0, 0; 3	+	+	*se* wo	5, 1, 8, 1; 15	+
+	*cn*	3,* 1,* 0, 0; 4	+	+	sr *sed*	2, 3, 0, 0; 5	+
+	g^2	1,* 0, 0, 0, 1	+	+	tk sf^2 abb	1, 0, 1, 2; 4	+
+	jv Hn^r h	4, 1, 1, 6; 12	+	+	*st*	1, 1, 1, 0; 3	+
+	*lt* c	2, 1, 0, 0; 3	+	+	*v*	3, 2, 5, 2; 12	+
+	*ma*	2, 2, 5, 7; 16	+	+	*w*	6, 0, 0, 0; 6	+

* Sex of donor not determined.

‡ Host dissected as mature pupa.

this paper, the data on this point are presented in table 1. These data show that most of the eye color mutants are autonomous in their pigmentation. The only clearly exceptional cases are those of *v* and *cn*. When implanted in wild type or in heterozygous *v*, the pigmentation of both of these is that characteristic of a wild type eye. In the case of *bo*, the result is not clear because the visible difference between an implant with *bo* pigmentation and one with wild type pigmentation is very slight. This is also true of the two eye color types as seen in normal eyes. Special experiments using other mutants as "intensifiers" of the difference between *bo* and wild type will probably be necessary to determine the behavior of *bo*.

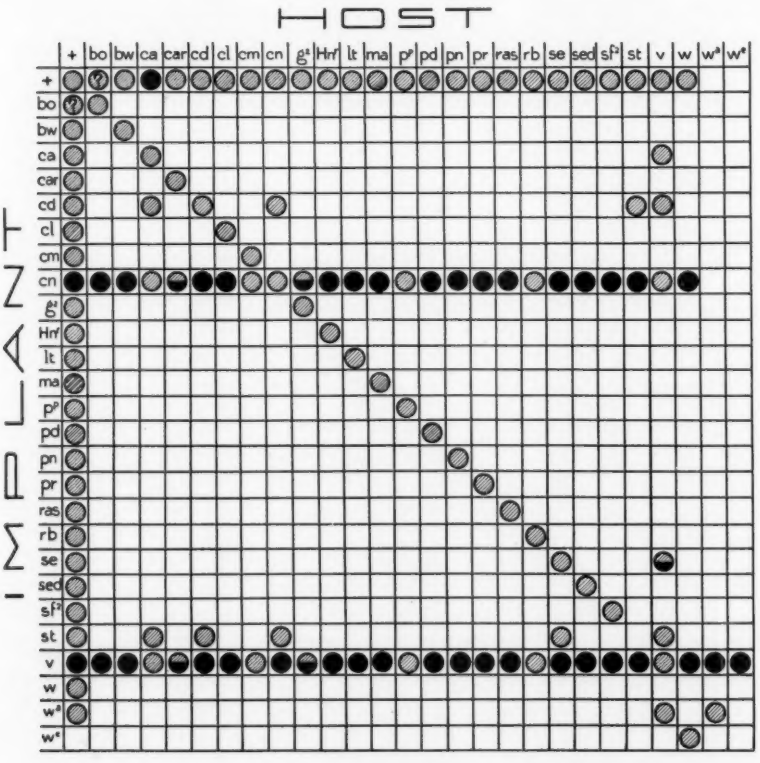

FIGURE 1.—Diagrammatic representation of the results of eye transplants. Shaded circles indicate autonomous development of the pigmentation of the implant. Black circles indicate non-autonomous development of pigmentation. Circles half black and half shaded indicate non-autonomous development of such a nature that the resulting implant is intermediate in color between two controls.

Wild type discs in mutant hosts

Knowing the behavior of the various mutant eye color discs implanted in wild type hosts, the reciprocals of these offer points of interest. The data are summarized in table 2.

It is evident that a wild type disc gives rise to an eye with wild type pigmentation when implanted in any of the mutants except *ca* and possibly *bo*. As in the reciprocal transplant, the result with *bo* is not clear. The significance of this exceptional behavior of + in *ca* transplants will be discussed later.

Vermilion discs in mutant hosts

In the case of a *v* disc implanted into a wild type host, the developing eye is affected by the host in such a way that the final pigmentation is like that of a wild type eye. Before discussing the factor responsible for this change in more detail and its relation to the factor responsible for the fact that a *cn* eye disc implanted into a wild type host develops wild type pigmentation, data should be considered which bear on the question of whether other eye color mutants have anything to do with this "body-to-eye" phase of the *v* reaction. This question can be answered by implanting *v* eye discs into hosts which differ from wild type by various eye color mutants. Such data are given in table 3.

TABLE 3

Data on the differentiation of v eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
v	*bo*	0, 0, 0, 1; 1	+	*v*	*pd*	2, 1, 1, 0; 4	+
v	*bw*	7, 0, 2, 1; 10	+	*v*	y *pn*	2, 0, 1, 1; 4	+
v	*ca*	4, 4, 3, 2, 3,* 2*; 18	*v*	*v*	b *pr*	2, 0, 1, 1; 4	+
v	*car*	5, 0, 2, 1; 8	Interm.	*v*	sc *ras*	0, 1, 0, 1; 2	+
v	*cd*	2, 1, 4, 1; 8	+	*v*	*rb* cv	2,‡ 1, 1, 0; 4	*v*
v	*cl*	2, 3, 1, 0; 6	+	*v*	*se* wo	4, 4, 6, 1; 15	+
v	*cm*	7, 0, 1, 2; 10	*v*	*v*	sr *sed*	0, 1,‡ 0, 0; 1	+
v	*cn*	6, 1, 3, 3; 13	+	*v*	tk sf^2 abb	3, 0, 0, 1; 4	+
v	g^2	2, 0, 3, 2; 7	Interm.	*v*	*st*	3, 0, 3, 2; 8	+
v	jv Hn^r h	1, 0, 2, 1; 4	+	*v*	*w*	8, 2, 2, 1; 13	+
v	*lt* c	2, 0, 0, 0; 2	+	*v*	w^a	0, 0, 0, 1; 1	+
v	*ma*	3, 0, 0, 3; 6	+	*v*	w^e	0, 1, 0, 0; 1	+
v	p^p	3,* 2,* 0, 0; 5	*v*				

* Sex of donor not determined.

‡ One host dissected as mature pupa.

These data show that, when implanted in certain mutant hosts (*bo*, *bw*, *cd*, *cl*, *cn*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and *w*), a *v* optic disc

gives rise to a wild type eye; in others (*ca*, *cm*, p^v, and *rb*), it gives an eye with *v* pigmentation. In *car* and g^2 hosts, a *v* disc gives an eye with pigmentation intermediate between *v* and wild type. Discussion of these relations will be deferred until other evidence is considered.

Cinnabar discs in mutant hosts

Since a *cn* disc implanted in a wild type host gives a result of the same type as the comparable implant of a *v* disc, namely, a wild type eye, the same question arises concerning *cn* as the one stated above for *v*. Data showing the results obtained by implanting *cn* eye discs in eye color mutant hosts are given in table 4. The results, excluding *cn* and *v* hosts, are the same as those for *v*, that is, a *cn* disc gives a wild type eye in the same mutant hosts in which a *v* disc gave a wild type eye, and gives a *cn* eye in the same hosts in which a *v* disc gave a *v* eye. Table 3 shows that a *v* disc in a *cn* host gives a wild type eye. Table 4 shows that the reciprocal transplant does not give this result, that is, a *cn* disc in a *v* host gives a *cn* eye.

TABLE 4

Data on the differentiation of cn eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
cn	*bo*	1, 1, 2, 0; 4	+	*cn*	*pd*	1,‡ 0, 5, 1; 7	+
cn	*bw*	3, 0, 0, 3; 6	+	*cn*	y *pn*	3, 0, 0, 0; 3	+
cn	*ca*	2, 0, 1, 0; 3	*cn*	*cn*	b *pr*	0, 1, 2, 0; 3	+
cn	*car*	2, 0, 5, 3; 10	Interm.	*cn*	sc *ras*	0, 0, 0, 1; 1	+
cn	*cd*	3, 3, 2, 2; 10	+	*cn*	*rb* cv	1, 0, 2, 1; 4	*cn*
cn	*cl*	2, 0, 1, 2; 5	+	*cn*	*se* wo	3, 3, 2, 3; 11	+
cn	*cm*	1, 2, 3, 1; 7	*cn*	*cn*	sr *sed*	0, 3, 2, 0; 5	+
cn	g^2	5, 1, 1, 2; 9	Interm.	*cn*	tk sf^2 abb	3, 0, 3, 0; 6	+
cn	jv Hn^r h	1, 0, 3, 0; 4	+	*cn*	*st*	3, 2, 2, 2; 9	+
cn	*li* c	0, 0, 2, 1; 3	+	*cn*	*v*	2, 4, 5, 1; 12	*cn*
cn	*ma*	1, 2, 1, 1; 5	+	*cn*	*w*	0, 2, 1, 0; 3	+
cn	p^p	5,* 3,* 0, 0; 8	*cn*				

* Sex of donor not determined.

‡ Host dissected as mature pupa.

Experiments concerning v, cn, and ca

From the data present above, it is seen that, in the cases of *cn* in wild type, *v* in wild type, and wild type in *ca*, the developing eye implant is influenced in its pigmentation by something that either comes or fails to come from some part or parts of the host. Just what this is, whether or not, for example, it is of the nature of a hormone, we cannot yet say. We shall therefore refer to it by the noncommittal term "substance."

Certain obvious questions at once arise concerning the substances con-

cerned in these three cases. For example, is there only one substance? If not, are the different substances related and in what way? What is their relation to the genes concerned in their production? Before attempting to discuss these and related questions, we shall consider additional data which bear on the problem.

Behavior of v in combination with other eye color mutants

By studying the differentiation of pigment in implants which differ from the host tissues by two eye color characters, one autonomous, the other non-autonomous in development, it might be possible to learn something about the interaction of the genes concerned. Data of this nature are summarized in table 5A for the combinations of *v*, $w^a v$ and *v car*. It is seen that the behavior of *v* is here the same as that observed in transplants in which *v* is the only mutant gene concerned. Likewise, *car* and w^a behave in the same way as in simple transplants involving only these mutant genes. This result tells us only that, so far as its behavior in transplants goes, the interaction of the *v* allelomorph with *car* or w^a plus the normal allelomorphs of all the other genes concerned with eye pigmentation is not different from its interaction with car^+ or w^+ under the same conditions. The same kind of result was observed by STURTEVANT (1932) in studies of early cleavage mosaics in *D. simulans* in which the individuals were made up of $v^+ g^+$ and *v g* tissue; here the *v* character is, under certain conditions, not autonomous, but the *g* character is always autonomous.

The relation of Bar and vermilion

In studies of the differentiation of Bar (*B*) eye discs implanted in not-*B* hosts, it was observed that a v^+B disc implanted in a *v* host gives rise to a *B* eye[1] with *v* pigmentation.

This experiment was repeated several times varying both the *v* stocks used as hosts and the *B* stocks which furnished the implants. The result was in all cases the same, indicating that the *B* gene, in addition to influencing the size of the eye in a characteristic way, has an effect closely related to the *v* reaction. The data from the various experiments involving the *v* and *B* mutants, as well as appropriate controls are given in table 5B. It is seen that only in case the host is *v*, does the *B* implant develop *v* pigmentation. An eye disc heterozygous for the *B* gene implanted in a *v* host gives an eye with wild type pigmentation. It follows that, whatever its action may be, the *B* gene effect is recessive in this interaction with *v*. These results suggested that the condition of some process in the *B* eye

[1] It is clear that a *B* disc implanted in a not-*B* host is *B* but whether or not there is any modification of the *B* character such as is observed in mosaics (STURTEVANT, 1932), we have not yet determined.

TABLE 5

Data on various eye implants. Explanations in text.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
		PART A	
sc *v* f *car*	+	14, 7, 4, 2; 27	*car*
+	sc *v* f *car*	1, 0, 2, 0; 3	+
sc *v* f *car*	*v*	2, 2, 1, 0; 5	*v car*
v	sc *v* f *car*	2, 0, 4, 1; 7	*v*
sc *v* f *car*	*cn*	1, 3, 3, 2; 9	*car*
$w^a v$	+	0, 0, 1, 1; 2	w^a
+	$w^a v$	6, 4, 2, 0; 12	+
$w^a v$	*v*	0, 0, 1, 1; 2	$w^a v$
v	$w^a v$	0, 2, 0, 4, 6,* 3*; 15	*v*
$w^a v$	*cn*	0, 0, 2, 0; 2	w^a
cn	$w^a v$	4, 5, 1, 2; 12	*cn* (host eyes w^a)
$w^a v$	wa^3	0, 0, 7, 0; 7	w^a
		PART B	
B	y *v* f	0, 0, 3, 0; 3	*v B*
B	*v*	2, 0, 1, 1; 4	*v B*
g^2 f *B*	*v*	0, 0, 3, 2, 5	*v* g^2B
B/+	*v*	3, 2, 0, 0, 5	*B*/+
B	+	0, 0, 3, 0; 3	*B*
B	*B*	0, 0, 0, 1; 1	*B*
se wo	*v*	6, 1, 5, 5; 17	*se* Interm. *v* (sex diff.-text)
		PART C	
cd	*cn*	2, 0, 0, 0; 2	*cd*
cd	*st*	3, 2, 6, 2; 13	*cd*
cd	*v*	2, 0, 3, 0; 5	*cd*
st	*cd*	0, 4, 1, 3; 8	*st*
st	*cn*	4, 3, 2, 3; 12	*st*
st	*v*	3, 0, 2, 0; 5	*st*
st	*se* wo	3, 0, 1, 0; 4	*st*
B	*st*	0, 0, 1, 2; 3	*B*
g^2 f *B*	*st*	0, 0, 1, 0; 1	g^2B
se wo	*cn*	4, 1, 1, 1, 7	*se*
cd	*ca*	3, 2, 5, 1; 11	*cd*
st	*ca*	1, 1, 0, 0; 2	*st*

* Sex of donor not determined.

disc at or after the time of transplantation might be retarded relative to the state of other developmental reactions, and led to experiments in which eye discs from young wild type larvae were implanted in older *v* larvae.

In table 6 data are given from transplants of this kind. In the first experiment, only two transplants were successful in the sense that the implanted discs gave rise to differentiated eyes. Here the age difference

between implant and host, at the time of transplantation, was about 28 hours. One of the two implanted eyes showed *v*-like pigmentation, the other more nearly wild type pigmentation. Unfortunately, in this experiment, there were no satisfactory controls. Later, an experiment was made in which young wild type discs were implanted in older *v* larvae, and at the same time, for a control, wild type discs of the same age and from the same culture dish of larvae were implanted in older wild type larvae. The data (table 6) show that, with an age difference of about 28 hours, the wild type discs implanted in *v* hosts did indeed give eyes with pigmentation approaching in color that of control *v* in *v* implants. The wild type in wild type controls with a similar age difference gave eyes with pigment of the same type as did known wild type control implants. In all cases the young discs implanted in older hosts gave rise to eyes markedly smaller than implanted eyes from transplants where little or no age difference exists between implant and host.

TABLE 6

Data on the differentiation of wild type eye discs from young larvae implanted in older v larvae. Arrangement under heading "Number of individuals" same as in previous tables.

IMPLANT		HOST			
CONSTITUTION	AGE AFTER HATCHING (HRS.)	CONSTITUTION	AGE AFTER HATCHING (HRS.)	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
+	44 to 48	*v*	80±	1, 1, 0, 0; 2	♀ *v* (?) ♂ + (?)
+	43 to 46	*v*	80±	3, 1, 3, 1; 8	Interm. between + and *v*
+	44 to 47	+	80±	4, 0, 1, 0; 5	+

From the data so far discussed, it might be assumed that the difference between *B* in *v* and wild type in *v* transplants is determined merely by the smaller size of the *B* implants. The behavior of young wild type implants in older *v* hosts could then be interpreted in the same way. But there are two arguments against this interpretation. In the first place, we have often obtained, from wild type in *v* transplants where there was no age difference, small fragments of eyes resulting from breakage of the disc during the operation of transplantation. In all cases these "small eyes" had wild type pigmentation. Many of these fragments were smaller than the eyes obtained in the "young in old" transplants. Furthermore, it is known from mosaics that small patches of v^+ tissue in an otherwise *v* eye have wild type pigmentation (STURTEVANT, unpublished). The second argument is one from analogy with the behavior of *se* in *v* transplants discussed below, in which there was little or no age difference between implant and host, but in which the implanted eyes were intermediate between *v* and v^+ (actually intermediate between *se* and *v se*, since *se* is autonomous in its

development). Here the implanted eyes were "normal" in size since the *se* gene does not affect eye size.

Actually, then, it appears probable that the behavior of *B* in *v* implants will find its explanation in terms of the states of certain eye reactions, influenced by the *B* gene, relative to the states of certain developmental reactions in other parts of the organism. Such a situation can, of course, following GOLDSCHMIDT, be expressed in terms of rates of certain eye reactions relative to the rates of other developmental reactions. What the nature of this eye reaction (or reactions) might be, we have, at present, no way of knowing. We shall return later to a consideration of its possible relation to the action of the *v* gene.

The experiments on *se* in *v* transplants mentioned above are summarized in table 5B. Actually these data are the result of three separate experiments, all of which gave the same result. Two *se* stocks were used, the second obtained by outcrossing the first to a *v* stock and recovering *se* flies in the backcross to the *se wo* stock. There was a definite difference between eyes developed from implants of discs from male and female donors; the male discs gave eyes with pigmentation more closely approaching *v se* control implants (*v se* in *v se*) than did female discs. Speculation concerning this effect of *se*, which may be of the same kind as the effect of *B*, will be more profitable when more data are at hand. The nature of the observed sex difference also needs further investigation.

Influence of eye implants on host eye pigmentation

In the above experiments in which $w^a v$ stocks were used, it was observed that $w^a v$ flies in which implanted *cn* eyes had developed, had normal eyes with w^a rather than $w^a v$ pigmentation. Since the $w^a v$ stock used had been recently made up, it was at first thought that this stock might not be pure. However, the same experiment was later repeated with adequate controls and the same result obtained. A *cn* eye implant, then, furnishes something to a $w^a v$ host fly which changes the course of eye pigment formation in such a way that the result is, in effect, v^+ and not *v* pigmentation. Since no such action of wild type or *cn* eye implants on the normal eyes of *v* hosts had been observed previously, a series of transplants was made to check this point carefully. The results were as follows:

Implanted eye disc	Host	Pigmentation of Implant	Pigmentation of Host
+	*v*	+	*v*
cn	*v*	*cn*	*v*
v	*v*	*v*	*v*
+	$w^a v$	+	$w^a v$
cn	$w^a v$	*cn*	w^a
$w^a v$	$w^a v$	$w^a v$	$w^a v$

These results suggest two obvious questions. The first is, why are the eyes of a $w^a v$ host changed by *cn* eye implants to w^a (from v to v^+) while the eyes of a v host are unaffected by such an implant? This change in the $w^a v$ eyes seems to be complete in many cases, that is, the modified eyes show no difference from stock w^a flies. Hence it seems clear that the same proportionate change does not occur in the two cases, detectable in $w^a v$ and not in v hosts. A more probable interpretation assumes that a *cn* eye implant releases into the blood of the host a certain quantity of some substance, presumably the same as that which changes the pigmentation of a v implant in a wild type host, and that this substance is only sufficient in amount to result in the change of a limited amount of pigment from v to v^+. The $w^a v$ eyes have little pigment and this can all be changed, by the available substance, from v to v^+. The normal eyes of a v host, on the other hand, have such a large amount of pigment that the limited supply of substance does not produce a detectable change, even though it may result in a change of the same absolute amount of pigment as in the case of the eyes of a $w^a v$ host. This interpretation obviously can be tested by relatively simple experiments. In fact, we have already observed that, in case the *cn* eye implant is small, the change in $w^a v$ is not complete.

A second question that is apparent from these results is, why is a *cn* eye implant effective whereas a wild type implant has no effect? Both types of eye implants of course have the v^+ gene, and presumably the production of v^+ substance goes on in both. It seems from the data that the *cn* gene produces a change such that the substance in question is released from the implant.

In connection with the influence of an eye implant on the eye color of the host, it is known, from studies of w^+-w gynandromorphs in *D. simulans* (DOBZHANSKY 1931; STURTEVANT 1932), that rate of testis sheath pigmentation is correlated with the amount of w^+ eye tissue present. The substance responsible for the pigmentation of the testis sheath very probably is formed by w^+ eye tissue—if so, it must be able to diffuse from the eye.

Implantation of gonads

In his studies of v-v^+ early cleavage mosaics in *D. simulans*, STURTEVANT (1932) was able to demonstrate clearly a strong correlation between the autonomous or non-autonomous pigmentation of genotypically v eye tissue and the constitution of the gonads with respect to the v gene. Here, if both gonads are v^+ (and female), genetically v eye tissue show v^+ pigmentation in practically all instances. If, on the other hand, both gonads are v (and male), genetically v eye tissue shows v or intermediate pigmentation in all cases. We have pointed out in a preliminary paper (EPHRUSSI and BEADLE 1935a) that it is the constitution of the gonads

with respect to the *v* gene and not with respect to sex that is important. In these experiments of STURTEVANT, there were some exceptions which led him to conclude that, in addition to the gonads, some other organ or part of the fly must be involved in the differentiation of *v* eye tissue in mosaics.

On the basis of STURTEVANT'S results we have made transplants of wild type ovaries in *v* hosts to see whether we could influence the pigmentation of the eyes of the host. Such ovary implants develop quite normally and are even capable of forming functional connections with the oviducts of the host (EPHRUSSI and BEADLE 1935b). The results of such experiments with ovary transplants, and which bear on the *v* case, are summarized in table 7.

TABLE 7

Data on transplants of non-v ovaries to v hosts.

CONSTITUTION		NUMBER OF DEVELOPED IMPLANT OVARIES	NUMBER OF INDIVIDUALS		PHENOTYPE OF HOST
IMPLANT	HOST		FEMALE	MALE	
+	*v*	1	17	7	*v*
ca	*v*	1	4		*v*
+	$w^a v$	1	29	5	$w^a v$
cn	$w^a v$	1	1		$w^a v$
+	*v*	2	5	2	*v*
+	*y v f*	3	2		*v*

It is seen that one or two wild type ovaries in a *v* male host or one, two, or even three such ovaries in a *v* female host, have no detectable effect on the *v* color of the eyes of the host. Likewise, neither an implanted wild type nor an implanted *cn* ovary has any influence on the normal eyes of a $w^a v$ host, male or female. These results, then, are entirely negative. Since in all these cases normal *v* ovaries or testes were present in the host, it could be argued that they account for the fact that implanted ovaries are without effect on the host eyes. However, this seems rather improbable as it would involve the assumption that the implanted ovaries produce the necessary substance but that something else produced by either *v* ovaries or *v* testes acts as an inactivating agent on the v^+ substance.

Taken in connection with the results of STURTEVANT which show quite definitely that wild type ovaries do have something to do with the production of the substance which changes the course of pigment formation in *v* eye tissue, our results only corroborate his conclusion that some other organ or part of the body plays an essential role in the production of this substance, i.e., gonads plus an unknown part of the body interact in its formation. Our studies give no clue as to what this unknown might be,

but STURTEVANT has shown that it is not closely related in terms of cell lineage to any surface part of the body, and does not lie in the abdomen (1932).

These results of gonad transplantation in Drosophila show certain obvious differences from those obtained by CASPARI (1933) and KÜHN, CASPARI and PLAGGE (1935) in gonad transplants in *Ephestia kühniella*, likewise made in connection with studies on eye pigmentation. These workers have shown that wild type testes or ovaries implanted in larvae of the red-eyed mutant race *a*, modify the eye pigmentation toward wild type. Here, then, the substance concerned, which they refer to as a hormone, can evidently be formed by the gonads from a wild type race in the absence of other organs or tissues of a^+ constitution. In this case, the substance has an effect on pigmentation in several parts of the organism, in larval skin, larval eyes, eyes of the imago, and in the gonads themselves. The substance can evidently be produced in other parts of the body since a wild type brain implanted in an *a* host modifies, under certain conditions, the pigmentation of the host.

Special experiments with the v-like group of mutants

The four mutants, *v*, *cn*, *st*, and *cd*, are very much alike in their phenotypic appearance. Furthermore, SCHULTZ (1935) has shown that in the development of their pigmentation, they show rather marked similarities and, as a group, are distinct from other mutants. In fact, on the basis of these similarities, he was led to suggest that they might all be found to show the *v*-type of behavior in mosaics. It has already been shown that, although *v* and *cn* are not autonomous in their pigment development in certain kinds of transplants, *st* and *cd* do show autonomous development in eye transplants in wild type hosts. Because of the similarity of *st* and *cd* to each other and to *v* and *cn*, we have used them in certain transplants in which other mutants have not been used (table 5). These data need little discussion. It is evident that both *st* and *cd* show autonomous development of pigment in all the combinations in which they are involved.

It is clear that the *v*-like group of mutants is not homogeneous as regards developmental behavior. In this respect *v* and *cn* are obviously related but not the same, as will be pointed out in more detail below, and *st* and *cd* are different from either *v* or *cn*.

The ca case

As shown by the data already referred to, a wild type eye disc implanted in a *ca* host gives an eye with *ca*-like pigmentation. To account for this result, we must assume that in the development of wild type pigment something must come to the eye from another part or other parts of the

body and that this substance is not formed in a fly homozygous for the *ca* gene. But the data given in tables 3 and 4 show that a *v* eye disc implanted in a *ca* host gives a *v* eye, i.e., not *v ca*, therefore ca^+, and that, similarly, a *cn* disc implanted in a *ca* host gives a *cn* ca^+ eye. Data given in table 5 show that a similar result is obtained if a *st* or a *cd* disc is implanted in a *ca* host, namely, a *st* ca^+ or a *cd* ca^+ eye results. Summary of these results:

+	disc implanted in a *ca* host gives a *ca* eye
v	disc implanted in a *ca* host gives a *v* ca^+ eye
cn	disc implanted in a *ca* host gives a *cn* ca^+ eye
st	disc implanted in a *ca* host gives a *st* ca^+ eye
cd	disc implanted in a *ca* host gives a *cd* ca^+ eye

In determining that the last four of these results were really *v*, *cn*, *st* and *cd* and not *v ca*, *cn ca*, *st ca*, and *cd ca* respectively, the appropriate double recessive controls were not available, but comparisons were made with *v*, *cn*, *st*, and *cd* control transplants and no differences could be detected. Since *v ca* and *st ca* are both known to be readily separable from *v* and *st* respectively, there is little chance of error in the determinations. The question, of course, is, why is the development of ca^+ pigmentation not autonomous in the first case listed and autonomous in the remaining cases studied? Possibly the four genes *v*, *cn*, *st* and *cd* act, in the implant, in such a way that no ca^+ substance is necessary to give ca^+ pigmentation; that is, a v^+ca^+ implant requires ca^+ substance from the host to develop ca^+ pigmentation, but a *v* ca^+ implant does not require this substance to develop *v* ca^+ pigmentation.

DISCUSSION

From the experimental results considered above, several hypotheses can be suggested concerning the nature of certain of the eye color mutants and the action of the genes which differentiate them from wild type. Alternative hypotheses are obviously possible, and it should be emphasized that those presented are tentative.

The vermilion character

Since the pigmentation of a genetically *v* eye can be modified to v^+ by transplanting it to a host which supplies it with what may be called the v^+ substance, it follows that *v* differs from wild type by the absence of this substance. Evidently there is no change in the *v* eye itself which prevents its pigmentation from assuming wild type characteristics. It follows that the mutation $v^+ \rightarrow v$ has resulted in a change such that v^+ substance is no longer formed. Since a *cn* eye disc implanted in a *v* host remains *cn*, the $v^+ \rightarrow v$ mutation has resulted also in preventing the formation of cn^+ sub-

stance. The v^+ gene plays an essential part in the formation of the v^+ and cn^+ substances, but it does not form them directly since any one of several other gene mutations (*ca*, *cm*, p^p, and *rb*) may result in the absence of them. According to this scheme, v^+ substance is necessary for wild type pigmentation. The question then arises, why does a wild type eye disc implanted in a *v* host, which can supply no v^+ substance, develop wild type pigmentation? Two answers are possible: either the v^+ substance has already acted at the time of transplantation or this substance is produced by the eye itself. The fact that, in mosaics, a small patch of v^+ eye tissue in an effectively *v* individual has wild type pigmentation (STURTEVANT, unpublished), shows that the first of these answers cannot be correct, for in this case, the v^+ tissue has been in a *v* tissue environment almost from the beginning of development. We must then conclude that the substance is produced in the eye itself. Actually we have been able to demonstrate that it is produced by a *cn* eye (modification of normal $w^a v$ eyes by an implanted *cn* eye). But, it may be asked, why was it not possible to demonstrate that it is produced by a wild type eye? The answer may be that the substance is produced but cannot get out of the eye, i.e., one of the effects of the *cn* gene is to make eye cells permeable to v^+ substance. The difference in behavior between a wild type and a *B* eye implanted in a *v* host may be accounted for by assuming that one of the effects of the *B* gene is to prevent the formation of v^+ substance in the eye, but not in other parts of the body. This assumption is not necessarily an alternative to the assumption previously suggested that the action of the *B* gene may be explained "in terms of the states of certain eye reactions, influenced by the *B* gene, relative to the states of certain developmental reactions in other parts of the organism." It may well be that it is the formation of v^+ substance that is retarded (in an extreme way) in the eye relative to its formation in other parts of the body. The "young in old" experiments can be formally explained in the same terms. In young wild type discs implanted in older *v* larvae, the time during which v^+ substance can be formed in the implanted eye is much reduced. In a similar way, in a *se* eye, v^+ substance is formed in the eye at a rate so low that, when implanted in a host without v^+ substance, pigmentation intermediate between v^+ and *v* results.

The cinnabar character

The evidence for the existence of a cn^+ substance is the same in kind as that for v^+ substance. It is already evident and will be pointed out in more detail below that the cn^+ substance is different from the v^+ substance. By the same kind of arguments as were presented in the above discussion of the *v* character, it may be concluded that the mutation $cn^+ \rightarrow cn$ produces

a change such that cn^+ substance is no longer formed. According to this interpretation, as in the interpretation of *v*, it is assumed that a wild type eye produces cn^+ substance in its own cells. This would account for the fact that a wild type eye implanted in a *cn* host gives wild type pigmentation.

The claret character

In contrast to the *v* and *cn* cases, two phases of the action of the *ca* gene can be distinguished. First, since a genetically wild type eye cannot develop wild type pigmentation unless some other part of the organism is ca^+, it is concluded that a ca^+ substance is necessary for the formation of wild type pigmentation. This is not formed in the eye itself but comes from some other part of the body. Secondly, since by supplying a *ca* eye with the necessary ca^+ substance by implanting it in a wild type host, we do not produce a change to wild type pigmentation, it is postulated that there is a change in a *ca* eye of such a kind that the addition of ca^+ substance is not sufficient to give wild type pigmentation.

Other eye color mutant characters

By implanting *v* and *cn* eye disc in other eye color mutant hosts, it has been demonstrated that the mutants *cm*, p^p, and *rb* are characterized by lack of both the v^+ and cn^+ substances. In these three mutant types, as in *ca*, there must be two phases of gene action, (1) the failure of the formation of the v^+ and cn^+ substances, and (2) an action in the eye itself, since supplying the two substances by transplantation does not produce a change. The genes *car* and g^2 must be placed in the same class, but in these two cases the formation of v^+ and cn^+ substances is not prevented but only limited.

The other mutants with which we have worked, *bo*, *bw*, *cd*, *cl*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and w^a are characterized by the presence of all the three substances postulated. It cannot be concluded that the normal allelomorphs of the genes differentiating these characters have nothing to do with the production of v^+, cn^+ and ca^+ substances. There is no justification in assuming that, if a given gene concerned with the production of a substance such as we are considering, mutates, the particular mutant allelomorph resulting will be of such a nature as to result in the absence of the substance. KÜHN, CASPARI and PLAGGE (1935) come to such an unjustified conclusion with regard to the t^+ gene in Ephestia.

Relation of the v^+, cn^+ and ca^+ substances

It has been shown from the difference in reciprocal transplants between *v* and *cn* that the v^+ and cn^+ substances are different (BEADLE and EPHRUSSI 1935a). At the same time, it was concluded from the fact that a *v* fly lacks

both substances, that the two substances are related. This conclusion is corroborated by the more extensive data presented in this paper. The strongest indication that two substances are concerned is the fact that a *v* eye disc implanted in a *cn* host gives rise to an eye with wild type pigment. Two other facts strengthen the supposition of two substances: (1) A *B* eye disc implanted in a *v* host gives an eye with *v* pigmentation, but, implanted in a *cn* host, gives wild type pigmentation. (2) A *se* eye disc implanted in a *v* host gives a *se*, partially *v*, eye, but, implanted in a *cn* host, gives a straight *se* eye.

The fact that these substances, although not the same, are developmentally—and presumably chemically—related, is shown by the fact that, if a given mutant is characterized by the absence of one of these substances, it will probably be characterized by the absence of the other also.

Considering the relation of the ca^+ substance to the other two, it is clear that it is different from either for it may be present in the absence of both the others. The fact that the *ca* gene prevents the formation of all three substances (*v* or *cn* discs implanted in *ca* hosts are not modified in their pigmentation) indicates that ca^+ substance is related to the other two.

It may be asked whether, from the relations discussed above, anything can be inferred as to (1) how the v^+, cn^+, and ca^+ substances are related in terms of development, and (2) how the mutant forms of the genes known to be concerned with the production of the three substances produce their effects? A simple, and, it seems to us, plausible, hypothesis may be of help in answering these questions. Such an hypothesis assumes that the ca^+, v^+, and cn^+ substances are successive products in a chain reaction. The relations of these substances can be indicated in a simple diagrammatic way as follows:

$$\rightarrow ca^+ \text{ substance} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance}$$

In such a scheme, we assume that:

1. The mutant gene *ca* in some way produces a change such that the chain of reactions is interrupted at some point prior to the formation of ca^+ substance; hence a *ca* fly lacks ca^+, v^+, and cn^+ substances.

2. Any one of the mutant genes *v*, *cm*, p^p, or *rb* results in a change such that the reaction or reactions leading from ca^+ substance to v^+ substance do not go on; hence the mutants *v*, *cm*, p^p and *rb* lack both v^+ and cn^+ substances but have ca^+ substance. The mutant genes *car* and g^2 slow down this step in the chain of reactions, hence *car* and g^2 flies are characterized by a reduced amount of v^+ and cn^+ substances. The mutant gene *B* interrupts this same step in the chain in the eye, but not in other parts of the body. The mutant gene *se* results in a change such that the ca^+ substance changes to v^+ substance at a reduced rate in the eye, but at a normal rate in other parts of the body.

3. The mutant gene *cn* stops a reaction essential for the change of v^+ substance to cn^+ substance; hence a *cn* fly lacks cn^+ substance but has the ca^+ and v^+ substances.

On the basis of the above scheme, the results of implanting *v* eye discs in *cn* hosts can be interpreted as follows: The implant produces no v^+ substance, and, because v^+ substance is an essential step in the formation of cn^+ substance, it likewise produces no cn^+ substance. The host can supply v^+ substance to the implant but cannot supply cn^+ substance. With v^+ substance supplied to the implant by the *cn* host, there is no block to the formation of cn^+ substance in the implant itself. The implant therefore develops wild type pigmentation in spite of the fact that normally neither the donor nor the host could have produced the cn^+ substance presumably necessary for the production of wild type pigment.

In a somewhat similar way, the results of transplanting *B* eye discs to *v* and to *cn* hosts can be interpreted. The *B* eye can form no v^+ substance. When transplanted to a *v* host v^+ substance cannot move to it from the host and the pigment developed is therefore *v*. Because of the absence of the prerequisite v^+ substance, the *B* eye normally does not itself produce cn^+ substance. But when a *B* eye disc is implanted in a *cn* host, the *B* implant is supplied with v^+ substance from the host and the reaction or reactions from v^+ to cn^+ substances can then go on in the implant itself and wild type pigment is produced.

The results of implanting *se* eye discs in *v* and *cn* hosts can be interpreted in an essentially similar way.

Eye color mutant groups

The eye color mutants in Drosophila can be grouped according to their phenotypic characteristics, since mutants differentiated by non-allelomorphic genes can look alike (MORGAN, BRIDGES, and STURTEVANT 1925). Recently SCHULTZ (1935) has extended this grouping by studying the time of appearance and the rate of formation of pigment, the distribution of pigment in the eye, and the interaction behavior of the different mutants. It is obvious that we can, on the basis of the results given above, classify the mutants with respect to the presence or absence of the three postulated substances. We may then ask if there is any relation between groups such as made by SCHULTZ and the classification according to these substances. If there is such a relation, it is not evident from the data at hand. As an example, the four mutants, *v*, *cn*, *st*, and *cd*, form one of SCHULTZ's groups but as we have seen, *v* lacks two substances, *cn* one, while *st* and *cd* have all three.

The above discussion, we hope, has served to indicate some of the possibilities in the application of the method of transplantation to the study

of development in Drosophila. The extension of the studies of certain cases to other stages of development is indicated as a logical next step by which we can hope to get at such questions as concern the time of determination of characters and the time of action of genes associated with these characters.

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SUMMARY

Larval optic discs can be successfully transplanted from one larva to another. Such transplanted discs give rise to supplementary eyes, usually lying in the abdominal cavity of the adult fly, which differentiate normally except that they are inverted. The pigmentation of such eyes develops normally.

When optic discs of the mutants *cn* or *v* are implanted in wild type hosts, they give eyes with wild type pigmentation, i.e., under these conditions, the *cn* and *v* characters are not autonomous in their development. Under the same conditions, *bw*, *ca*, *car*, *cd*, *cl*, *cm*, g^2, Hn^r, *lt*, *ma*, p^p, *pd*, *pn*, *pr*, *ras*, *rb*, *se*, *sed*, sf^2, *st* and *w* eye discs implanted in wild type hosts show autonomous development of eye pigment.

In the reciprocals of the above transplants, wild type eye discs implanted in hosts of the mutants mentioned, wild type pigmentation of the implant results in all except one case, a wild type disc implanted in a *ca* host. In this one exception, a genetically wild type eye disc gives an eye with *ca* pigmentation, i.e., ca^+ does not show autonomous pigment development under these conditions.

If *v* eye discs are implanted in eye color mutant hosts, eyes with wild type pigmentation develop in *bo*, *bw*, *cd*, *cl*, *cn*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and *w* hosts, i.e., the *v* character is not autonomous in its development when a *v* eye is transplanted to any one of these hosts. But a *v* eye disc implanted in a *ca*, *cm*, p^p, or *rb* host gives an eye with *v* pigmentation, i.e., the *v* character is autonomous in these cases. It can be concluded that the autonomous or non-autonomous development of the

v character is determined by the genetic constitution with regard to genes other than *v*, of the tissue environment in which the *v* eye develops.

Implanted in eye color mutant hosts other than *v* or *cn*, a *cn* eye disc behaves in the same way as does a *v* eye disc, showing autonomous pigment development in the same mutant hosts as does *v*, and non-autonomous pigment development in the same hosts as does *v*.

Reciprocal transplants involving *cn* and *v* do not give the same result; a *v* eye disc implanted in a *cn* host gives an eye with wild type pigmentation while a *cn* eye disc implanted in a *v* host gives an eye with *cn* pigmentation.

A *B* eye disc implanted to a *v* host gives a *B* eye with *v* pigmentation. This shows that the *B* gene has an effect on the eye somehow related to the effect of the *v* gene but not of such a nature as to modify the pigmentation of the eye in its normal position. This case shows that the autonomous or non-autonomous development of v^+ pigmentation in an implanted v^+ eye may be influenced by the genetic constitution, with respect to genes other than *v*, of the implant itself.

A genetically wild type eye disc from a young larva implanted in an older *v* host shows pigmentation intermediate between *v* and wild type. A *se* eye implanted in a *v* host likewise gives pigmentation of an intermediate nature with respect to the *v* character; here the eye is intermediate between *v se* and *se*. The possible relation of these cases to the *B* in *v* results is considered.

A *cn* eye implanted in a $w^a v$ host gives a *cn* eye, but the eyes of the host are modified from the $w^a v$ to a w^a phenotype.

Ovaries from wild type donors have been implanted in both male and female *v* hosts without any detectable change in the pigmentation of the host eyes.

From the cases of non-autonomous development of the pigmentation of implanted eyes considered in this paper, three substances are postulated, the v^+, cn^+, and ca^+ substances. Their interrelations and the conditions under which they are produced are discussed. A hypothetical scheme accounting for the production and relation of these three substances is suggested, and, in connection with this, questions concerning where and how certain genes might act are considered.

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THE DEVELOPMENT OF VESTIGIAL WINGS UNDER HIGH TEMPERATURE IN DROSOPHILA MELANOGASTER

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INTRODUCTION

THE PRESENT paper deals with three questions concerning the development of vestigial wings in Drosophila: First, where is the critical period during which the development of vestigial can be affected by high temperature (31°), the so-called temperature-effective period? Second, to what extent can the wing form be modified by temperature? And lastly, is the enlargement of the wing as a result of the temperature treatment due to the increase of size or number of cells?

The work of HARNLY (1930, 1932 and 1933), STANLEY (1928, 1931 and 1935), HERSH (1932) and others has already covered a considerable amount of ground in the study of the vestigial and temperature relationship in Drosophila. The present work, however, represents the beginning of a slightly different approach to the question and is concerned with a more direct embryological study than has hitherto been attempted.

THE TEMPERATURE-EFFECTIVE PERIOD

A pure vestigial stock of *Drosophila melanogaster* originally obtained from Columbia University, New York City, was used for the following experiments. The food was prepared from local material according to the formula adopted for this laboratory (LI 1930). Only two temperatures were employed to treat the vestigial flies: 25° and 31°C. For the lower temperature, we have used the cabinet gas-burning type of incubator designed by BRIDGES and LI (1932); for the high temperature, the only incubator available was of the water-bath type. During the years 1932 to 1934 the following experiments were performed.

High temperature treatment of pupae

In a number of cultures five pairs of strong and vigorous vestigial flies were allowed to breed. The larvae were raised in the 25° incubator and, as soon as puparia were formed, they were isolated within an hour. About 30 pupae thus isolated were put on a strip of moistened blotting paper and raised through the rest of their life cycle in a vial with a small amount of food to keep the paper continuously moist. These vials were then divided

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into two batches: "A" and "B." The one-hour old pupae in the vials of "A" series were allowed to start their development at 31°, but at each of the succeeding 6-hour intervals, were transferred to 25° for the rest of their life cycle. In the vials of the "B" series the pupae were allowed to begin their development at 25° and were subsequently transferred at the same time intervals to 31°. The purpose of the reciprocal transfers in the experiment was to narrow down from both ends of the pupal period the temperature-effective period for the high temperature. As controls, four cultures were tested simultaneously with the others. First, both larvae and pupae were raised at 25° (X1, table 3); second, the larvae were raised at 25°, but the pupae at 31° (X2); third, both larvae and pupae were raised at 31° (Y1); and lastly, the larvae were raised at 31°, but the pupae at 25° (Y2).

When the adults emerged from the pupal cases, they were etherized and their wings were measured under a binocular microscope with a standard micrometer. As a rule only the right wing of each fly was measured. However, when the latter was injured or folded, the left wing was then taken as a substitute, provided both wings were of approximately equal lengths. The results of the experiment are summarized in the following tables:

TABLE 1

The mean lengths in mm of vestigial wings of flies beginning their pupal period at 31° and transferred to 25° at the intervals indicated.

HOURS AT 31°	♀♀		♂♂	
	M	N	M	N
6	0.81	19	0.74	19
12	0.77	36	0.69	20
18	0.70	33	0.69	21
24	0.73	19	0.63	17
30	0.73	27	0.67	19
36	0.74	24	0.66	27
42	0.75	28	0.69	37
48	0.76	17	0.68	36
54	0.80	26	0.70	25
60	0.75	18	0.70	26
66	0.79	27	0.68	24
72	0.73	24	0.66	27
78	0.77	30	0.74	27

The results given in tables 1 and 2 show that the mean lengths of the vestigial wings of the treated flies are fairly uniform, which means that there is no particular period in the whole pupal stage that responds to high temperature treatment. Comparing with the 25° control (X1, table 3), it can be further shown that there is no significant increase in wing length as a result of high temperature. If there is any effect at all, high tempera-

TABLE 2

The mean lengths in mm of vestigial wings of flies beginning their pupal period in 25° and transferred to 31° at the intervals indicated.

HOURS AT 25°	♀♀		♂♂	
	M	N	M	N
6	0.68	15	0.64	11
12	0.71	15	0.61	22
18	0.79	19	0.68	17
24	0.76	17	0.70	21
30	0.73	20	0.72	30
36	0.78	19	0.72	42
42	0.78	36	0.71	25
48	0.79	20	0.74	25
54	0.78	28	0.73	32
60	0.82	17	0.72	25
66	0.79	30	0.69	24
72	0.86	28	0.71	21
78	0.83	27	0.75	30

TABLE 3

The mean lengths in mm of wings of all vestigial flies raised in both "A" and "B" series and in the controls.

SERIES	♀♀		♂♂	
	M	N	M	N
"A"	0.75±0.01	328	0.69±0.01	325
"B"	0.78±0.01	291	0.71±0.01	325
X_1	0.80±0.01	132	0.74±0.01	129
X_2	0.78±0.01	12	0.71±0.01	15
Y_1	1.06±0.04	32	1.63±0.05	25
Y_2	1.05±0.01	124	1.45±0.02	122

ture tends to make the vestigial wings slightly shorter when the pupae are subjected to it, as indicated in table 3. When we compare the mean lengths of the vestigial wings of all the flies in both "A" and "*B*" series and those of the controls X_1 and X_2, it can be noted that when the pupae were raised at 31,° the wing length of the fly is consistently shorter than otherwise. The results in table 3 also show that when the larvae were raised at 31°, irrespective of treatment of the pupae, there is a great increase in wing length (Y_1 and Y_2, table 3). It follows therefore that a much more significant temperature-effective period is to be found in the larval period.

High temperature treatment of larvae

In order to locate this critical period in the larval stage by means of reciprocal transfers the larvae of various ages were exposed to 31°. This would naturally necessitate careful isolation of the newly hatched larvae,

as it has been shown that there is a considerable amount of variation in the time of hatching of eggs isolated at the same time (LI 1927). For this purpose a large number of vials was prepared, in each of which a glass slide with a piece of blotting paper of corresponding size was placed. The blotting paper was first soaked in fermented banana juice and then a thin layer of banana agar food was carefully spread on it. Five pairs of strong parental vestigial flies were allowed to remain on the food while eggs were laid during a 6-hour interval in the 25° incubator. Slides with eggs on them were taken out of the vials and examined one by one under a binocula. The newly hatched larvae within the interval of one hour were isolated with a fine scalpel and transferred to a culture bottle, fifty larvae in each bottle. Thus they were all about one hour old and allowed to develop under almost identical conditions of food and amount of space. The culture bottles in duplicates were then treated as in the case of pupae in the earlier experiments, that is, reciprocal transfers between two temperatures 25° and 31°. As soon as the larvae pupated the bottles were all removed to 25° to let the flies finish the rest of the life cycle. Tables 4 and 5 give the results of the wing measurements of the treated flies.

In working through the data shown in the above two tables one may note that there is a great variability in the wing measurements of the individual flies as indicated by the high value of the coefficient of variabilities (C.V., tables 4 and 5). The percentages of mortality in most cases

TABLE 4

Mean lengths in mm of vestigial wings of flies beginning their larval period at 31° and transferred to 25° at the intervals indicated.

HOURS AT 31°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
6	0.85±0.01	9.53	21	0.72±0.02	9.40	12	38.91
12	0.89±0.02	14.18	23	0.82±0.01	7.02	19	22.21
18	0.86±0.01	9.69	20	0.75±0.01	9.33	11	42.58
24	0.83±0.01	10.32	23	0.78±0.01	9.39	19	22.21
30	0.85±0.02	11.87	19	0.73±0.01	8.22	31	57.70
36	0.86±0.01	12.71	33	0.73±0.01	7.45	53	21.75
42	0.88±0.02	15.10	31	0.75±0.01	9.00	32	51.75
48	0.92±0.02	11.01	22	0.78±0.01	7.28	38	44.38
54	0.86±0.01	10.26	26	0.86±0.01	14.90	43	37.75
60	0.93±0.01	10.05	35	0.89±0.03	22.85	29	40.75
66	1.02±0.02	10.94	24	0.90±0.01	6.39	23	12.96
72	0.99±0.02	10.48	20	0.90±0.03	16.22	15	35.20
78	1.09±0.01	16.18	17	1.49±0.05	22.21	18	35.20
84	1.05±0.03	23.30	28	1.23±0.05	30.28	29	47.20
90	1.12±0.03	20.08	33	1.44±0.06	28.30	19	51.83
25° control	0.80±0.01	15.08	132	0.74±0.01	11.21	129	—
31° control	1.06±0.04	25.87	31	1.63±0.05	22.41	25	—

TABLE 5

Mean lengths in mm of vestigial wings of flies beginning their larval stage at 25° and transferred to 31° at the intervals indicated.

HOURS AT 25°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
6	—	—	—	—	—	—	100.00
12	0.95±0.04	10.41	14	1.64±0.06	17.20	9	57.49
18	0.94±0.02	11.62	23	1.47±0.05	14.97	8	42.60
24	0.93±0.02	9.21	14	1.55±0.03	10.70	12	51.75
30	1.03±0.03	17.90	25	1.33±0.04	26.49	29	50.00
36	0.90±0.02	10.72	19	1.20±0.04	23.70	24	20.38
42	0.92±0.02	14.40	33	1.23±0.06	36.85	28	43.51
48	1.06±0.04	28.81	33	1.04±0.04	29.50	32	39.80
54	0.91±0.02	12.31	22	0.82±0.01	13.57	41	41.70
60	0.93±0.01	11.83	31	0.88±0.03	20.42	20	53.72
66	0.83±0.01	15.00	47	0.81±0.01	15.45	43	16.67
72	0.92±0.03	16.91	14	0.77±0.01	8.82	17	42.55
78	0.74±0.01	8.43	21	0.72±0.01	8.74	25	14.82
84	0.77±0.01	12.82	45	0.66±0.01	11.50	40	21.30
90	0.71±0.01	9.93	50	0.65±0.01	10.80	40	16.68
25° control	0.80±0.01	15.08	132	0.74±0.01	11.21	129	—
31° control	1.06±0.04	25.87	31	1.63±0.05	22.41	25	—

are very high, especially when the larvae were first started in 25° and later transferred to 31°. These coupled with the small number of individuals measured made it hazardous to draw any definite conclusions as to the effect of high temperature on the wing development of the vestigial flies. However, as indicators of the time at which high temperature begins to exert its influence on the development of vestigial wings and when this influence ceases, the data do show certain things. In cases where the larvae started their development at 31° and then were transferred to 25° (table 4), it can be noticed that at about the sixtieth hour there is a decisive tendency for the vestigial wings to become longer. This point must be taken as the beginning of the temperature-effective period for 31°C. In the case of the opposite transfers (table 5) it is also easily seen that beginning with the seventy-eighth hour, the mean length of the vestigial wings becomes steadily shorter. This must mean that the temperature-effective period ends before the latter point is reached. On the basis of these observations we may fix the temperature-effective period for vestigial wings roughly at sixty to seventy-two hours in the larval period, making a total of approximately 12 hours. The data further show that at 31°, with the stock of vestigial flies used, there is a distinct sexual-dimorphism in the wing lengths such as observed by the earlier workers (ROBERTS, HARNLY and STANLEY).

In order to overcome some of the difficulties in variability and mortality encountered in the above experiments, a number of similar tests were made. In these later attempts, we used twice as many flies from a vestigial stock that had been inbred for nine generations. Another deviation in procedure from the earlier experiments was that after they had been isolated the larvae were allowed to develop for the first 24 hours at 25° without any disturbance, at the end of which, they were all transferred to 31°, except of course the 25° controls. From 31° at each of the succeeding 6-hour intervals, the larvae were again transferred to 25° to complete their development. No transfers in the opposite direction were made.

The reason for allowing all the larvae to pass the first 24 hours in 25° instead of 31° is primarily an attempt to reduce mortality of the larvae. Since the temperature-effective period is found to be approximately in the latter half of larval life, the elimination of the first day of high temperature treatment should not interfere with the result as far as the location of the critical period is concerned. At the same time, it may be of interest to see whether such elimination would affect the development of the vestigial wings. In order to test this point, two 31° controls were employed. In one of these, vestigial flies were raised throughout their larval stage at 31° (control 2, table 6), while in the other, the larvae were first exposed to 25° for one day like the rest of the cultures in the experiment and then were transferred to 31° (control 1, table 6).

Table 6 gives the results of one such experiment. It can be seen that

TABLE 6

Mean lengths in mm of vestigial wings of flies, kept at 25° for the first 24 hours of their larval period, raised at 31° and subsequently transferred to 25° again at the intervals indicated.

PERIOD AT 31°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
24–30	0.81±0.01	11.09	67	0.74±0.01	12.12	56	38.5
24–36	0.81±0.01	12.59	86	0.73±0.01	15.92	88	13.0
24–42	0.79±0.01	9.38	85	0.74±0.01	9.37	69	23.0
24–48	0.81±0.01	10.62	81	0.73±0.01	8.87	76	21.5
24–54	0.80±0.01	9.13	81	0.74±0.01	9.36	73	23.0
24–60	0.85±0.01	12.05	65	0.84±0.01	6.02	62	36.5
24–66	1.02±0.03	21.60	87	1.07±0.04	29.03	89	12.0
24–72	1.07±0.03	19.82	75	1.16±0.04	21.85	64	30.5
24–78	1.01±0.02	13.61	69	1.16±0.04	24.42	57	37.0
24–84	0.92±0.02	13.12	64	0.99±0.03	25.30	78	29.0
24–90	0.99±0.02	15.16	54	0.99±0.04	26.50	65	15.5
25° cont.	0.79±0.01	13.12	77	0.72±0.01	8.94	98	12.5
31° cont. 1	1.03±0.04	25.32	81	1.28±0.04	26.10	84	17.5
31° cont. 2	1.73±0.05	30.40	74	1.77±0.05	21.01	32	47.0

beginning with the sixtieth hour of larval life, the lengths of the vestigial wings of the treated flies show a distinct increase, especially in the males. In the females the increase in lengths of wings is not very striking until the sixty-sixth hour interval. It is possible that by starting the larvae at 25° for the first day, the whole developmental process may be somewhat slowed down, as compared with those which were raised entirely at 31°, thereby causing a slight delay in the onset of the critical period. The slight drop in the mean wing lengths after the seventy-eighth hour interval may not be very significant. It should not be interpreted as the end point of the temperature-effective period; without data on the opposite transfers, such an end point cannot be determined with certainty.

Regarding the increase of wing length of the flies exposed to high temperature, one may note that the striking difference in wing lengths between the sexes is very much reduced if not entirely gone. This probably means that with inbred stock, more or less homogeneous in its make-up, the so-called sexual-dimorphism can be eliminated and in both sexes the development of vestigial wings then responds to high temperature equally well. However, when the first day of the larval period was not subjected to high temperature, neither sex of the vestigial flies could have wing lengths comparable to those whose whole larval period was subject to it. This is clearly shown in the two 31° controls (controls 1 and 2, table 6). So in order for the vestigial wings to reach the maximum length, the first 24 hours of the larvae must be treated with high temperature (in this case 31°). It follows therefore that high temperature at this particular period supplements the process involved in the enlargement of the wings. This supplementing process however could do nothing unless the larvae were continuously treated under high temperature during the critical period (60–72 hour interval).

The same point can be verified from yet another experiment. When only short intervals of 12 (60–72 hours) or 24 (48–72 hours) hours from the latter half of the larval period of the vestigial flies and covering the whole length of the temperature-effective period were subjected to 31° the average lengths of the wings of either sex could not be increased to more than 1.03 ± 0.01 mm (from unpublished data). In view of these facts, any assumption that the increase in size of the vestigial wings is due to high temperature treatment only at the critical period may be held as questionable.

THE FORMS OF THE WINGS OF THE TREATED VESTIGIAL FLIES

As we examined the wings of the treated vestigial flies at the time when they were measured, we noted that there was a great deal of variation in the forms of the wings. Greatest variation was found in flies longest ex-

posed to 31° and in the 31° control. After having made all the necessary measurements, we fixed these flies in 95 percent alcohol. Wings were taken off from the flies very carefully with fine forceps under a binocular and dehydrated by passing through the diaphane solvent and finally mounted with diaphane. Drawings were made under the Edinger Drawing apparatus according to the same scale of enlargement.

The regular vestigial flies are not capable of flying but those raised in high temperature may have wings enlarged enough to fly almost as well as the wild type. However, their wings are comparatively thinner than those of the wild types and therefore are easily folded and injured. Balloon formation by the separation of the upper and the lower surfaces of the wings either partly or completely was of common occurrence especially when the flies were fixed in weaker alcohol.

In the untreated vestigial wings (figures 1 and 2), it can be shown that except the marginals, all the longitudinal veins of a typical Drosophila wing are present and easily identified under a binocular microscope. A short axillary vein accompanied by a humeral crossvein is also present. The first three longitudinal veins are branched from the main stem which is divided very distinctly by two transverse sutures. The fourth, fifth and the smaller sixth longitudinal veins are developed very irregularly. The anterior and posterior crossveins together with anal crossvein are entirely absent. Among the vestigial flies the wing forms are not at all uniform, but as a rule the wings look folded and thickened, apparently due to the contraction of the longitudinal veins.

For the sake of description the various wing forms produced by high temperature treatment are conveniently classified into five types as follows:

Type 1 (figures 3 and 4): These are similar to the untreated regular vestigial wings. However by careful study, one may note four differences: First, the edges are more or less invaginated at the point where the longitudinal veins end; second, the posterior and anal crossveins are usually present; third, the marginal hairs are developed in places along the costal and the first longitudinal veins; fourth, the wings are slightly longer and broader than the untreated vestigials.

Type 2 (figures 5 to 14 inclusive): In type 2 there are two subdivisions (a) and (b), one of which is obviously derived from the other.

(a) The wings are longitudinally well extended but slender and pointed at the distal end. The marginal cell is present as a narrow strip. The anterior and anal crossveins are well developed; and the second longitudinal vein is usually fully extended sometimes even bearing marginal hairs. The latter may also develop at the region in front of the distal end of the first longitudinal vein (figures 5, 6 and 7).

(b) The wings are similar to (a) except the distal end of the wing turns upward and is often enlarged and somewhat rounded (figures 8, 9, 10 and 11).

Type 3 (figures 12, 13 and 14): The wings are broader than those of type 2 and the distal end is somewhat blunter and broader than the proximal end. The posterior crossvein is usually present or at least shows a rudimentary development.

Type 4 (figures 15 to 20 inclusive): Wings in this case show the marked development of the submarginal and the second posterior cells to present the general appearance of a fork. The posterior crossvein is always present. Sometimes the submarginal cell, the second posterior and the third posterior cell are especially extended to form two or three projections at the distal portion of the wing.

Type 5 (figures 21, 22 and 23): In this type of wing, all the typical wild-type characteristics are present with the one exception that the margin may be cut or notched in one or several places. The wings are usually attached to the body of the fly in the same manner as that of the wild-type.

It must be noted that while type 1 and type 5 described above undoubtedly represent two extremes and conceivably the latter might have been resulted by an all round expansion of the former, one cannot be sure that the intermediate types (types 2, 3 and 4) belong to a progressive series from the narrow to the broad types of wings. On the contrary, they may have occurred quite at random. The similarity of these forms to strap, antlered and various other allelomorphic phenotypes has already been pointed out by earlier workers (STANLEY 1931, etc.).

It is interesting to note that in the vestigial wings of the untreated flies, all the longitudinal veins of the wild-types are present. The enlargement of these vestigial wings as a result of temperature treatment is accompanied by the extension of these longitudinal veins. The posterior crossvein appears only when the wing reaches a certain breadth and the finishing touches of the wing development are the marginal hairs.

Besides the types mentioned, there are several other peculiarities observed, such as wings which appear as a triangle or like a tongue and sometimes an extra crossvein parallel with the anterior crossvein may be developed. Perhaps the most remarkable fact of all that we have observed is that in a number of cases the right and left wings of the same individual were strikingly different (figures 26 to 29 inclusive).

The size and number of cells of vestigial wings after high temperature treatment

Vestigial flies obtained from the experiment just described have furnished material for this study. The flies were first cooked with 10 percent

KOH and washed through a series of alcohols before the wings were taken off and mounted in diaphane, which would make the wing very transparent. For cell counting, we have adopted the method of DOBZHANSKY (1929). The work was done under the Edinger apparatus. The submarginal cell of the wing was arbitrarily chosen as the marked area for hair counting because of the several advantages. 1 The particular cell is present almost in all cases; 2 it is clearly defined by the second and the third longitudinal veins; 3 this particular cell is very sensitive to temperature treatment; and 4 the hairs here are comparatively regularly arranged and evenly distributed. Two kinds of counts were made: one on the hairs of the whole area of the cell and the other on those within a standardized small area inside of the cell. Specimens from ten of the males for each of the differently treated vestigial cultures were taken at random and thus examined. The results of these counts are shown in table 7.

TABLE 7

The average number of hairs in the submarginal cell etc. of the vestigial wings of flies raised at high temperature during the larval stage in the various periods indicated.

PERIODS EXPOSED TO 31°C.	NO. HAIRS IN SUBM. CELL	LIMITS	NO. HAIRS IN STANDARD AREA	LIMITS
24–30	135	40– 200	38.5	28–48
24–36	146	80– 250	37.8	28–43
24–42	246	90– 640	38.7	31–48
24–48	162	110– 250	36.7	31–41
24–54	214	130– 280	35.7	31–42
24–60	330	170– 580	32.7	25–39
24–66	799	400–1120	35.1	31–39
24–72	771	480–1100	32.1	26–38
24–78	827	600–1170	32.3	28–35
24–84	828	570–1020	30.6	24–35
31° cont.	1094	940–1200	30.2	28–32
wild-type ♂♂	1723	1680–1760	20.7	19–22

It is evident from the results shown in the above table that the number of cells in the submarginal cell was increased with the increasing length of exposure to high temperature, while a somewhat reversed situation is seen in the case of cell counts within the standardized area. There is also a wide range of variability in both counts, but the range gradually diminished as the flies were exposed to high temperature for longer periods. Comparing with the wild-type males, one finds that the average number of cells per chosen area is more constant and both the number and size of the cells in the submarginal cell of the wing are larger in the wild-type than any of the treated vestigials. While the increase in size of cells in

the wings of the treated vestigial flies may be due to a large extent to the stretching of the wings, the increase in number of cells must be the result of high temperature treatment. It is concluded therefore that the enlargement of the vestigial wings of the flies raised in high temperature is largely due to the increase in the number of cells.

Besides the experiment just described, we have also made a comparative study of the larval wing buds of (1) the vestigial flies (2) those under high temperature treatment and (3) the wild-type. As the results will be taken up in a separate paper, only a general statement will be made here. Beginning with the fifty-fourth hour of the larval period, the posterior part of the mesothoracic bud was seen to expand gradually. This change was more clearly seen in wild-type and in temperature-treated vestigials than in the untreated ones. From the sixtieth to seventy-second hour period the so-called wing bud begins to be formed from the posterior portion of the mesothoracic disc. The wing bud rudiments in wild-type and in treated vestigials are comparatively more pronounced than those of untreated vestigials. Comparing the time occupied by the temperature-effective period in the larval stage with that during which the changes of the imaginal discs occur, one may be led to conclude that high temperature at this critical period has an accelerating effect upon the growth of the mesothoracic bud and causes it to develop a larger wing bud in the vestigial flies.

DISCUSSION

It was ROBERTS (1918) who first discovered that there is a peculiar relation between high temperature and the development of the enlarged vestigial wings. He further showed that the critical period of temperature effect lies between the fertilization of the egg and the pupal stage. Among recent investigators along this line, HARNLY (1930, 1931 and 1933) and STANLEY (1928, 1931 and 1935) have done some very critical work. HARNLY (1930) found that there is a progressive increase in length of the vestigial wings with the progressive increase in temperature from 18° to 31°C. He later (1933) showed that the gradual enlargement of the vestigial wing under high temperature (30° 31° and 32°) follows a typical sigmoid growth curve and that the growth period extends from the sixtieth to eighty-fourth hour after the egg starts to develop.

Much detailed work has been done by STANLEY (1928 etc.) in locating the temperature effective period for such temperatures as 17°, 27°, 30° and 31° etc. Although both HARNLY and STANLEY started their experiments with only the age of eggs known, their data on the temperature-effective period for 31° are fairly comparable with what we found with the larva-isolation technique. It seems to us that the latter method of determining

the critical period has at least two advantages. In the first place, we can get a more exact knowledge of the mortality rate of the larvae and, secondly, a more accurate determination of the time interval in the larval period as such. According to our data, this critical period begins roughly at the sixtieth and ends at the seventy-second hour of the larval period. This particular period approximates to the time when the imaginal discs for wings are about to be differentiated from the mesothoracic buds (CHEN 1929).

It is possible, as shown in our tests, that high temperature in the pupal period may have some effect upon the size of the vestigial wing, causing slight reduction in the size (table 3). We have also found that the temperature in the first 24 hours of the larval life tends to accelerate the enlargement of the wings provided the flies are also exposed to it during the temperature-effective period. By raising the larvae from the beginning of the larval period at 31°, the vestigial wings may reach an average length of 1.73 ± 0.05 mm for the females and 1.77 ± 0.05 mm for the males. These figures are quite comparable to those which STANLEY found for the same temperature (STANLEY 1935). But when the larva was allowed to develop at 25° for the first 24 hours and then raised through the rest of the larval period at 31°, there was a considerable reduction in the mean lengths of the vestigial wings in both males (1.28 ± 0.04) and females (1.03 ± 0.04). It is of interest to note that according to CHEN (1929) the mesothoracic disc, from which the wing anlage is derived, appears during the first 24 hours of larval life.

The fact that different temperatures give different growth curves (HARNLY 1933) and that they have also different effective periods (STANLEY 1935) indicate that the situation is by no means a simple one. The complex situation is further shown by the high degree of variability in wing form. It becomes necessary that the whole problem should be looked into from a somewhat different angle. A study therefore from the embryological point of view may help to clear up some of the confusion. GOLDSCHMIDT (1935) in connection with his study of genes and external characters has examined the development of a series of mutant types affecting the form of the wing in Drosophila, such as vestigial-notch, cut, beadex, Beadex-Jollos and vestigial. By comparing the changes of the imaginal discs of the wings in the various mutations mentioned above in the pupal stage (from 8 to 30 hours), he found that although the wings of the adults of the above differ from one another, they all start in the early pupal period from imaginal discs comparable in size and pattern. Later however degeneration (Erkrankung) of the epithelial tissues of the wing buds sets in first from the tip, next the hind border and then the front margin, causing the wings to be remodeled according to the type of

genes affecting them. In the case of vestigial wing, the anlage according to GOLDSCHMIDT's findings is actually 2 to 3 times as large as the adult wing before the degeneration process sets in and makes it shrivel to produce the adult pattern.

GOLDSCHMIDT's study of the series of mutations from vestigial-notch to vestigial led him to infer that in the case of vestigial, the imaginal disc of the wing is somewhat comparable in size and pattern to that of the wild-type, but the degeneration of the epithelial tissue of the anlage starts much earlier (in the larval stage) than the others, such as cut, beadex and Beadex-Jollos, thereby giving rise to a much smaller and abnormal type of wing. Accordingly, he is inclined to interpret the temperature effect on the development of the vestigial wing in a different way from what has been implied by the earlier workers. According to GOLDSCHMIDT, the increase in size of the vestigial wing as a result of temperature treatment during the development of the fly is possibly due to the delay of the onset of the degeneration process rather than the direct increase in the size of the anlage.

Our work with the cell counts of the vestigial wings of flies raised under high temperature shows clearly that there is a decided increase in the number of cells. Unless the temperature effect is to prevent more cells from becoming degenerated, which is unlikely (since high temperature in the pupal period tends to decrease rather than increase the size of wings in the vestigial flies), it must be taken to mean that it has an accelerating effect in the growth period. It is unfortunate that GOLDSCHMIDT has not examined the imaginal discs of the vestigial flies in the larval period, so it is not possible to say, from his evidence to what extent the anlage of the vestigial wing is comparable to the other types. Our as yet unfinished work in connection with the comparative study of the imaginal discs of the mesothorax and the wing of larvae from wild-type, vestigial and vestigial raised at high temperature tends to show that these discs are larger in the case of the treated than the untreated vestigials. Without direct evidence at hand, we may have to infer that the more rapidly growing buds of the treated vestigials are the result of a higher rate of cell division in the formative period before the anlage starts to degenerate. These facts may somewhat modify the point of view held by GOLDSCHMIDT and they tend to show that the ultimate size and pattern of the vestigial wings of flies raised in high temperature is the result of at least two interrelated factors: the enlargement of the anlage of the mesothorax and wing on one hand and the delay of the onset of degeneration process on the other. Perhaps the high degree of variation in size, form and period of temperature effectiveness may all be explained when these two factors are carefully analyzed.

SUMMARY

1. Newly formed puparia of vestigial flies in Drosophila were isolated in vials and treated with the two temperatures 31°C and 25°C. The vials were divided into two series; the puparia in one of the series were first exposed to 31°C and then transferred in each of the succeeding six-hour intervals to 25°C until the end of the pupal stage; those of the other series were used as the opposite transfers.

2. The wing lengths of the vestigial flies so treated were measured and compared with the controls to see if the temperature had any effect on the flies. The results of the experiments tend to show that high temperature (31°C) is not effective in lengthening the wings of the vestigial in the pupal period. Possibly it may cause a slight reduction in the mean lengths of the wings.

3. However, when in like manner, the same temperatures were applied to the newly hatched larvae of the vestigial flies, the data on subsequent wing measurements showed that the high temperature was very effective in enlarging the vestigial wings. The temperature effective period is located at an interval which begins at approximately the sixtieth and ends at the seventy-second hour of the larval period.

4. By raising the first-day larvae in 25°C and then subjecting them to high temperature treatment described above, it was shown that the mean wing lengths of the vestigials so treated were significantly shorter than the cases when the first twenty-four hours of the larvae were also spent in high temperature. The evidence thus indicates that in order to realize the maximum lengthening of the wing characteristic of the temperature 31°C, not only the so-called temperature effective period, but also the first twenty-four hours of the larval stage must be exposed to the temperature.

5. The forms of the wings of the vestigial flies that have been exposed to 31°C for the whole (or almost whole) larval period showed a high degree of variability. This variation seems to bear no definite relationship with the sex of the individual or the size of the fly. Furthermore, the right and left wing of the same individual may occasionally be strikingly different.

6. By making various hair-counts of the submarginal cell of the wings of the treated flies, it is possible to show that the gradual enlargement of the wings as a result of high temperature treatment is primarily due to the increase in number rather than size of the cells.

7. The results of the above observations lend themselves to the interpretation that the high temperature tends to cause an increase in size of the mesothoracic and later the wing buds by increasing the rate of growth and cell division in these bodies and that the temperature seems to be particularly effective at the time when these buds start to form in the larval period of the fly.

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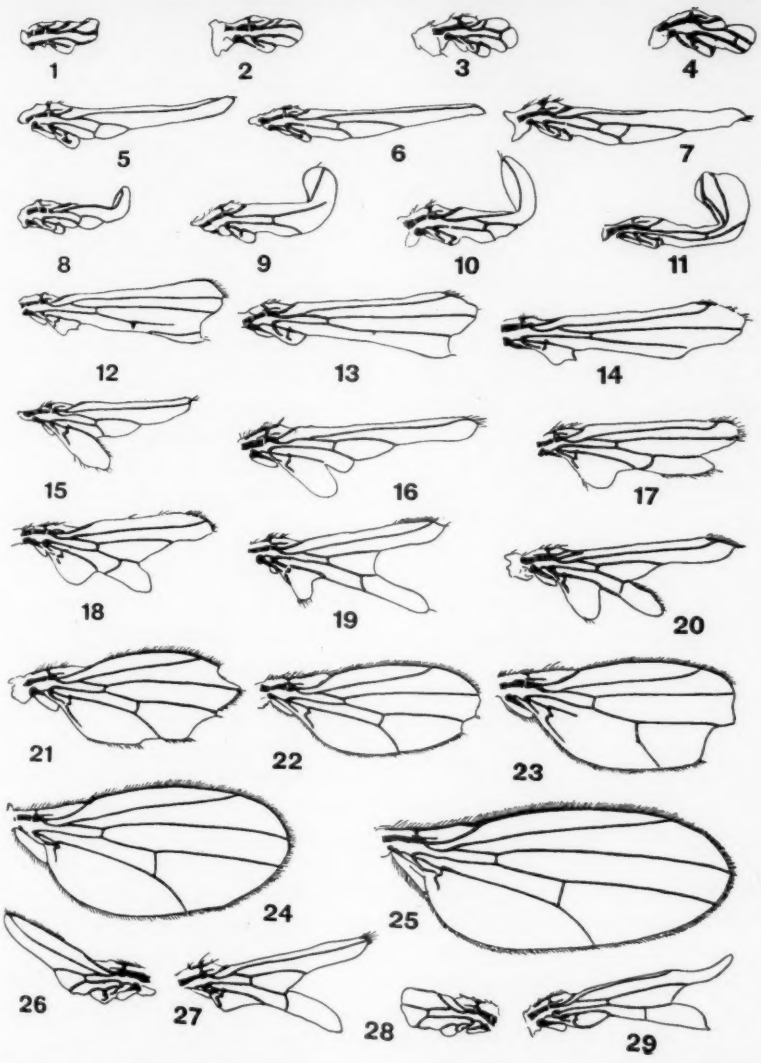

FIGURES 1, 2—Normal vestigial wings.
FIGURES 3, 4—Type 1 wing variations.
FIGURES 5–7—Type 2a wing variations.
FIGURES 8–11—Type 2b wing variations.
FIGURES 12–14—Type 3 wing variations.
FIGURES 15–20—Type 4 wing variations.
FIGURES 21–23—Type 5 wing variations.
FIGURES 24, 25—Wild type wings ♂ and ♀ respectively.
FIGURES 26, 27—Left and right wings in one individual.
FIGURES 28, 29—Left and right wings in another individual.

CYTOLOGIC STUDIES ON THE ABNORMAL DEVELOPMENT OF THE EGGS OF THE CLARET MUTANT TYPE OF DROSOPHILA SIMULANS

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INTRODUCTION

ALTHOUGH many instances of abnormal development of zygotes containing mutant genes have been described in the course of genetic studies on Drosophila, few attempts have been made to determine the mechanism of such development. Much of the material which has been studied has been poorly adapted to genetic analysis of this problem, since mutant genes usually produce developmental abnormalities so slight as to be difficult to identify, or so great as to produce complete inviability of the eggs containing such genes. The claret mutant gene of *Drosophila simulans* produces effects on the egg which are neither too great nor too small for study of this kind since some of these eggs develop into viable adults showing major abnormalities. For this reason genetic studies of the offspring of the claret mutant type have been completed, and have led to the suggestion of a probable mechanism for the production of the abnormalities found. A further study of this suggested mechanism may be made by the use of the cytologic method, basing such study on the genetic data at hand.

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REVIEW OF LITERATURE

In 1924, PLUNKETT found in a stock of *D. simulans* from Austin, Texas, a mutant fly with a claret eye color. Flies homozygous or heterozygous for this mutant gene were of good viability and normal in appearance, but homozygous females, on breeding, showed an additional abnormality. Although these females laid a normal number of eggs many of the eggs failed to develop, and those which did develop gave rise to adults only a few of which were normal. Females heterozygous for the mutant gene, and males both homozygous and heterozygous, produced the usual number of normal offspring. STURTEVANT and PLUNKETT, in 1926, located the claret mutant gene in the third linkage group, and found it to correspond to the claret mutant gene of *D. melanogaster*. The presence of the claret mutant

gene in *D. melanogaster* is not associated, however, with developmental abnormalities. STURTEVANT, in 1929, published the results of his genetic studies on the offspring of females homozygous for the claret gene. He described the kinds of abnormalities found among the viable offspring, and the frequency of occurrence of these abnormalities. He confirmed the observation of PLUNKETT that from the females it was possible to obtain only a very small number of viable eggs.

In addition to the high frequency of inviability, STURTEVANT found a second abnormality among the viable offspring of the claret females. This was the occurrence of flies whose sex-linked characters were unlike those expected from the matings made. Of the males produced by a cross, 50 percent were such exceptions, the sex-linked characters being apparently determined by the paternal rather than the maternal X chromosome. Of the females, 6 percent showed exceptional sex-linked characters. Such anomalous flies have been found in other stocks where they have been shown to be produced by failure of the X chromosomes to disjoin during the maturation of the egg. This process, however, would give rise to exceptional males and females in equal numbers rather than to such disproportionate numbers as STURTEVANT found. Similar disproportions of exceptional males to females had been observed earlier in *D. melanogaster* by BRIDGES (1916), SAFIR (1920), MAVOR (1924) and ANDERSON (1924) and in *D. simulans* by STURTEVANT (1921). These investigators suggested that the inequality between males and females might have been produced by occasional failure of the X chromosomes to reach either pole of the maturation figure, or by elimination of both maternal X chromosomes during the first cleavage. Either of these processes would produce only exceptional males while non-disjunction would produce both exceptional males and females. The fact that the inequality of males and females in the claret stock of *D. simulans* is greater than that found in other stocks was not explained by STURTEVANT.

A third type of abnormality found by STURTEVANT was the occurrence of flies having small bristles and frequent imperfections of the last section of the fourth vein of the wing. These "diminished" flies were shown by chromosome studies to be haplo-IV in constitution. Since no fourth chromosome mutant was available, it was not possible to determine whether the missing chromosome was that of the paternal or the maternal set.

In addition to these abnormal types, STURTEVANT found flies which gave evidence of similar abnormalities involving, however, only part of the tissues of the fly. This type of abnormality involved the X chromosome in 3 percent of the offspring, resulting in the production of gynandromorphs. The fourth chromosome was involved in 4 percent of the offspring, resulting in the production of "diminished mosaics" which were haplo-IV

in part of their tissues. The lack of a fourth chromosome mutant prevented further studies on the diminished mosaic flies.

From the complete studies of the sex-linked characters STURTEVANT found that two classes of gynandromorphs occurred among the offspring of the claret females. These classes may be described by a specific example. If a female, homozygous for the claret and yellow genes is crossed to a wild type male, the offspring are expected to be wild type females and yellow males. Exceptional yellow females and exceptional wild type males were also produced. The gynandromorphs produced by such a mating were of two classes. The first, including 90 percent of the gynandromorphs, consisted of wild type female tissue (phenotypically similar to the normal females produced by the mating) and wild type male tissue (similar to the exceptional males). The second class, including the remaining 10 percent, consisted of yellow female tissue (phenotypically similar to the exceptional females produced by the mating) and yellow male tissue, (similar to the normal males).

Additional observations which proved to be of importance in the formulation of a mechanical picture of the events causing the abnormalities of these eggs may be mentioned. In both the sex and fourth chromosome mosaics the abnormal tissues most often comprised ½, ¼, or ⅛ of the fly. More rarely fractions other than these were observed. Further, an abnormality of one linkage group in the whole or part of a fly was not necessarily accompanied by an abnormality of another linkage group. No major abnormality involving the second or third linkage groups was observed.

From these results, STURTEVANT set up the following hypothesis to account for the abnormalities found. Elimination of chromosomes may occur during the maturation divisions of the egg or during the early cleavages. Elimination of an X chromosome during maturation produces a female pronucleus deficient in its X chromosome, and fusion of this with a male pronucleus produces an XO male or an inviable YO zygote. (Exceptional females may be produced by a second process, non-disjunction). Elimination of a fourth chromosome during maturation produces a fly of haplo-IV constitution. If an X or a fourth chromosome is eliminated from one of the nuclei formed during the first cleavage, a fly is produced which is deficient for this chromosome in approximately half of its tissue. Elimination during later stages of cleavage produces flies which are abnormal in varying amounts, depending on the time of elimination of the chromosome. Elimination of a second or a third chromosome during maturation or the cleavage divisions produces an inviable zygote.

To account for the peculiar classes of gynandromorphs found, a further assumption was necessary. As indicated above 91 percent of the gynandromorphs had normal female and exceptional male tissue. If, according to

STURTEVANT's hypothesis, the exceptional male tissues are produced by the elimination of an X chromosome during cleavage, it must, in these cases, be the maternal X which is eliminated. This process produces a fly in which the male parts are XO in constitution, the remaining X chromosome being that of the paternal set. The assumption may also explain the second class of gynandromorphs, which may be produced by the elimination of a maternal X during cleavage, if such a chromosome is eliminated in an egg which began its development as an exceptional (XXY) female. The female tissue then remains exceptional while the male tissue, having suffered the loss of one of the X chromosomes, becomes similar genetically to the normal males expected from the same mating.

In other reported cases of the occurrence of gynandromorphs, no evidence of the selective elimination of maternal chromosomes has been found. Apparent evidence of such elimination was reported by BRIDGES (1925) in Minute-n *D. melanogaster*. But here elimination was of this type because the mutant gene caused elimination of the chromosome which carried it. If it had been carried by the paternal chromosome, that chromosome would have been eliminated (STERN, 1927).

In the cytologic study of the eggs of homozygous claret females STURTEVANT's hypothesis has been a valuable working guide, indicating as it does, the probable stages in development when abnormalities are likely to occur. However, if an attempt is made to apply the hypothesis as a mechanical picture of the possible cytologic events, the explanation becomes less simple than it appears at first. To picture a mechanism which will permit the loss, during cleavage, of chromosomes of the maternal set, and rarely or never, those of the paternal set, is difficult. For, with the completion of the first cleavage, the two sets of chromosomes lie together in close association. To drop those of one set selectively would require a peculiar and complex mechanism.

A new hypothesis for the production of gynandromorphs has therefore been set up on the basis of the cytologic data. This hypothesis, to be presented below, fits the genetic data, and in addition lends itself to the postulation of a simple cytologic mechanism for producing the abnormalities. Further, an attempt will be made to establish the validity of STURTEVANT's suggestions that chromosomes may be excluded from the female pronucleus as a result of the peculiarities of the first maturation division, and that this process, when it involves the second or third chromosomes results in failure of the egg to develop.

MATERIAL AND METHODS

To secure the eggs for study, a stock was used from which both normal and abnormal material could be selected. Since homozygous claret females

produce few viable eggs, the stock was maintained by mating heterozygous females with males homozygous for the mutant. For the study of the abnormal eggs, matings were made between homozygous claret females and heterozygous males. For control material, to show the normal course of development of *simulans* eggs, heterozygous females were mated to homozygous claret males. The stocks used were also homozygous for the third chromosome recessive, scarlet. This mutant gene had no appreciable effect on the viability of either the homozygous or heterozygous claret flies.

Flies were segregated as they emerged and fed on banana-agar mixture for five days before mating. Females were starved for several hours before collection of eggs was to begin, and permitted to mate. The wings of the females were cut off under ether to permit the posterior end of the abdomen to be observed during laying. For the collection of eggs such flies were placed in vials containing a glass slide on which was placed a piece of dark blotting paper moistened with fermented banana and yeast. During the period of laying these flies were kept at a temperature of 25.5 to 26.5° C.

To obtain abnormal and control eggs of comparable ages it was necessary to determine for each egg used the time interval between fertilization and fixation. In flies from which the wings have been removed, it is possible to see, after an egg has been laid, several protrusions of the uterus before the laying of the next egg. In the first few of these protrusions, the uterus appears yellow, translucent, and pointed at the posterior end. After several such protrusions the uterus, during one of them, becomes wider, and after another slight movement, becomes opaque, blunt at the end, and presents a shiny white tip. This tip is the posterior end of the egg, which has reached the end of the uterus. The anterior end of the egg is now in position to receive sperm. Fertilization was therefore assumed to occur when the white tip appeared at the end of the uterus, and timing of the eggs was begun at that moment. Slides made from control eggs timed in this manner showed similar stages of development when allowed to develop for similar lengths of time, justifying the timing procedure used.

The position of each egg on the blotting paper and the time of fertilization was recorded. After the desired time interval had elapsed, the slide was removed from the vial. The eggs were transferred to Kahle's formol-alcohol-acetic fixative and punctured at once with a sharpened steel needle (HUETTNER, 1923). To avoid distortion of the maturation figure which is found on the dorsal surface of the egg near the anterior end, eggs were punctured on the ventral surface, posteriorly. The puncture, when successful, permitted only a minute globule or a thin strand of oöplasm to escape.

Eggs were stained with erythrosin in 85 percent alcohol to facilitate orientation in paraffin under the binocular microscope. Sections were cut sagittally, 7μ in thickness and stained with Heidenhain's iron haematoxylin.

DESCRIPTION OF CYTOLOGIC PREPARATIONS

1. Control stock—development of normal eggs of D. simulans

The development of the normal control eggs follows closely that described by HUETTNER (1924) for *D. melanogaster*. With one exception the chromosomes of the two species are similar. The Y chromosome of *D. melanogaster* consists of one long and one short arm. The short arm is absent in the Y chromosome of *D. simulans*. The earliest fertilized eggs obtained were in the anaphase of the first maturation division. This figure lies, as in *D. melanogaster*, at the periphery of the egg, in a small, yolk free, cytoplasmic island on the dorsal surface near the bases of the filaments of the chorion. In eggs fixed 5 minutes after fertilization the chromosomes lie at the poles of the first maturation spindle (figure 1). No reorganized nuclei are seen at the completion of this period, the chromosomes lining up immediately on the spindle for the second maturation division. Stages of this division are found in eggs fixed between 5 and 10 minutes after fertilization (figure 2). Eggs which have developed for 11 minutes (figure 4), show the four reorganizing polar nuclei, three of which, as is true in *D. melanogaster*, remain in the cytoplasm at the periphery of the egg. One of the four nuclei lies nearer the center of the egg, where the sperm, not shown in this figure, is undergoing reorganization.

Preparations of eggs fixed during the next few minutes do not permit of identification of the reorganized egg nuclei which at this time stain poorly, and are again visible as clear vesicles only in preparations of eggs which have been permitted to develop for 15 minutes. Eggs prepared during the next 5 minutes show these four nuclei, each containing a haploid set of chromosomes. One of these vesicles, the female pronucleus, lies near the center of the egg, in the protoplasmic island containing the male pronucleus, wherein the haploid set of chromosomes is also visible. Material fixed from 20 to 23 minutes after fertilization shows the polar chromosomes lying free in the peripheral cytoplasm in three haploid groups, the nuclear membranes having disappeared. At this stage, two spindles are seen in association with the chromosomes of the male and female pronuclei. Figure 4 shows a portion of such a preparation, in which the three haploid groups of polar chromosomes may be seen. The cleavage figure present near the center of the egg is not shown. During later cleavages the two spindles characteristic of the first cleavage are no longer to be seen, the

maternal and paternal chromosomes being closely associated on a single spindle. Such late cleavages further indicate the persistence of the polar chromosomes or nuclei in the peripheral cytoplasm.

2. Homozygous claret stock—development of abnormal eggs of D. simulans

a. First maturation division. As in the control preparations, the abnormal eggs immediately after fertilization are in the anaphase of the first maturation division. The figures at this stage are distinctly abnormal, showing one general type of abnormality; namely, separation of the chromosomes at either end of an abnormally wide maturation spindle. Figure 5 shows such an abnormal figure. The chromosomes are greatly separated at one pole, less so at the other. Other preparations show modifications of this type of abnormality, the extreme being a separation of the chromosomes into several widely scattered groups as seen in figure 12. The spindles in these figures are correspondingly distorted.

b. Second maturation division. The abnormal eggs again differ from the control eggs at the time of the second maturation division. In the control stock, stages of the second maturation division are found in eggs prepared from 6 to 10 minutes after fertilization. Those of the abnormal stock of comparable age seldom show a second maturation division in progress, the chromosomes lying as they did at the end of the first maturation division. In some preparations the double nature of these chromosomes is already evident. During the next 5 minutes of development preparations show no evidence of the polar chromosomes, which lose their staining capacity as do those of the control preparations at the conclusion of the period of the second maturation division.

Fixation 15 minutes after fertilization discloses the presence of the vesicular nuclei formed by the reorganization of the chromosomes at the end of the maturation divisions. In contrast to similar preparations of control eggs, where four such nuclei are seen, the abnormal egg shows the presence of four to twelve reorganized nuclei.

c. Cleavage. In material fixed during the following period (20 to 30 minutes after fertilization) the chromosomes within the polar nuclei and pronuclei are visible. The chromosomes are long and thin, twisting about each other, thus making identification of the fourth chromosomes, the smallest of the set, impossible. The other chromosomes, however, are readily recognized. Where more than four of these egg nuclei are present, each contains less than the haploid number of chromosomes. The extreme of this condition is seen in those preparations where twelve nuclei are present, each containing but a single chromosome (figure 6).

The pronuclei in preparations of this age again show differences from those of the control preparations, where each of the pronuclei, lying side by side, showed the presence of its own spindle. In the abnormal stock

the male pronucleus occupies its normal position and contains a normal spindle. The female pronucleus may be abnormal in position and in spindle formation. One or several of the nuclei produced by the maturation divisions may lie in association with the male pronucleus, but in many cases all these egg nuclei lie at the periphery of the egg so that no one of them may be identified as a female pronucleus. Spindles are rarely seen in association with any of these egg nuclei. When the total number of such nuclei is twelve, no spindle is ever seen in association with the female pronucleus, even when this is normal in position. Many preparations with fewer nuclei also lack the spindle of the female pronucleus. Figures 7 and 8 show two unusual preparations in which spindles are present in both pronuclei. In each of these, the female pronucleus contains both the second and third chromosomes, figure 7 indicating further a lack of an X chromosome and figure 8 the presence of an extra X chromosome. In no preparation was the female spindle present when the female pronucleus lacked a second or a third chromosome.

The majority of the preparations at hand show no cleavage figure. Eggs fixed during the next few minutes may still show the presence of the sperm spindle, but this is lost in older preparations, the male pronucleus appearing again as an interkinetic, lightly staining vesicle. Still later preparations, where second and third cleavages figures are expected, show instead the picture of nuclear degeneration, the pronuclei and polar nuclei being irregular in outline and lightly stained. An increased number of polar nuclei is visible at this time, the number of nuclei approximately doubling as the period for each unrealized cleavage passes. Spindles are not seen in association with this nuclear increase.

Those preparations which show evidence of normal cleavages following the first, still show the presence of polar chromosomes whose nuclear membranes have disappeared. These polar chromosomes lie scattered throughout the cytoplasm between the periphery of the egg and the centrally located protoplasmic islands containing the cleavage nuclei (figure 9). As in the more normal preparations, the number of these chromosomes increases at each cleavage.

d. Unfertilized eggs. It is important to determine the time of development at which the first sign of abnormality appears in the eggs of the claret females. Preparations were therefore made of eggs dissected from females before fertilization or laying. Figure 10 shows the earliest of these, in which the prophase of the first maturation division is seen. Figure 11 shows a later prophase figure. Both these preparations show no sign of the abnormality to follow. Figure 12 shows the first visible sign of the distortion of the maturation figure, groups of chromosomes being widely separated in the cytoplasm rather than in the single compact group characteristic of the control preparations at this stage of development.

THEORETICAL DISCUSSION

In the discussion to follow, consideration of the fourth chromosome will be omitted. This chromosome is small and is identified in normal eggs by its position in close association with the other chromosomes. In the abnormal preparations where the chromosomes are scattered, this relation is lost, and it becomes impossible to differentiate the small chromosome from nearby yolk granules of the same size.

The first abnormality which appears in the development of the eggs of the homozygous claret females is the distortion of the first maturation spindle and the separation of the chromosomes at its poles. The subsequent abnormalites of development may be explained as results of this initial abnormality. To carry out this explanation each of the abnormalites will be reviewed in the light of those which took place in the preceding stages of development.

The picture presented at the time of the second maturation division may be considered to consist of two distinct abnormalities. The first of these is the failure of the second maturation to occur in orderly fashion in most eggs. Spindles for the separation of the chromosomes at this time are rarely seen. During this period the chromosomes lie as they did at the conclusion of the first maturation division, three long chromosomes being visible at each pole of the spindle. The small fourth chromosome is difficult to identify. These six chromosomes disappear during the next few minutes, and on their reappearance are found to have increased to twelve. Thus the chromosomes have doubled during the period of the second maturation division, but this division was an abnormal one, unaccompanied by any spindle.

The second abnormality of this period becomes evident at the conclusion of the second maturation period, and concerns the formation of an abnormal number of polar nuclei, each containing fewer chromosomes than the haploid set. The chromosomes at one end or at both ends of the distorted first maturation spindle are often widely separated from each other. No immediate effect of this is visible since no nuclear reorganization follows this division. However, when nuclear reorganization does occur, at the completion of the period of the second maturation division, it is apparent that the widely separated chromosomes have not been able to enter in normal fashion into the organization of these nuclei. For, instead of forming a compact nucleus containing all the chromosomes at one pole of the spindle, these chromosomes, scattered widely from each other, form instead, separate nuclei, each containing only a single chromosome. If each of the four chromosomes in each of four haploid sets found at the end of the two maturation divisions were thus to form its own nucleus, sixteen nuclei would be found. Such is not the case, however, the largest number at this time being twelve. The small fourth chromosome does not form

its own nucleus, either remaining with one of the others or, if separated, failing to form a membrane about itself. This point could not be determined because of the difficulty of following the fate of the fourth chromosome.

The phenomenon of the formation of nuclei by single chromosomes has been called "idiomerie" by HAECKER and other workers, and occurs in both normal and experimentally treated tissues. POLITZER (1934) has reviewed the early work in this field. STRASBURGER (1880) and HAECKER (1895) described the formation, during normal mitosis of independent vesicles formed by each independent chromosome, these blending to form the typical single interkinetic nucleus. SCHILLER (1909) and TOBIAS (1914) were able to accentuate this slight degree of normal idiomerie by treatment of the eggs of Cyclops at high temperature. In all these cases the several vesicles fused finally to produce a normal interkinetic nucleus. ALBERTI and POLITZER (1934) so accentuated idiomerie by treatment with X-rays that the independent vesicles failed to fuse. It is this type of idiomerie that is found to occur in the untreated eggs of the claret mutant females under consideration.

The varying number of polar nuclei found in the abnormal eggs ready for first cleavage is the result of varying degrees of abnormality of the first maturation division. Complete separation of all the chromosomes at this time, with the exception of the fourth chromosome which apparently does not act independently, would produce twelve nuclei. Other variations in the number of these nuclei may be produced by partial scattering of the chromosomes of the first maturation division, separating two chromosomes from a third, or separating only those at one pole as in figure 5.

The next abnormality to be seen is the failure of cleavage in the greater number of eggs studied at this stage. Cleavage fails in several types of eggs. Such eggs include those in which twelve nuclei are present after the maturation divisions, none of these nuclei ever showing the formation of a maternal cleavage spindle. Also, eggs in which these nuclei are more nearly normal in number often fail to cleave. In the rare cases in which cleavage does occur, the female pronucleus is always found to contain at least the second and third chromosomes, in accordance with the genetic data presented by STURTEVANT.

We may further compare those chromosome groupings in the female pronucleus which permit of normal cleavage with others which do not. Figures 7 and 8 show two eggs in which the first cleavage is in progress, each of these eggs containing the diploid set of second and third chromosomes. Figure 7 indicates further a lack of an X chromosome in the female pronucleus and figure 8 the presence of an extra X chromosome, lying in its vesicle just outside the female pronucleus. Despite the X chromosome irregularities, cleavage is in progress. Figure 6, on the other hand,

shows an egg which has failed to cleave. Surrounding the normal male pronucleus are three of the twelve nuclei produced by the maturation divisions, these containing one of the second, third and X chromosomes. These three chromosomes, when associated with the male pronucleus in other eggs, were sufficient to permit of normal cleavage. Here, where the necessary chromosomes lie in separate nuclei, cleavage has not taken place. It is thus apparent that in order for normal cleavage to occur, the second and third chromosomes must reach the male pronucleus and that they must do so enclosed within a single nucleus. If they do not lie in a single nuclear membrane, no spindle is formed for their division, and cleavage fails. Figure 8 illustrates this further. Here a single female pronucleus containing the second, third and X chromosomes is functioning in cleavage. But an extra nucleus (one formed by the maturation divisions), lying above the female pronucleus and containing a single X chromosome, has formed no spindle and has been unable to enter the cleavage figure up to this time.

The first abnormality observed, that of the first maturation division, is thus again responsible for the abnormality found at this time. Moreover, this early abnormality has at last achieved the result of stopping any further development of the egg, by causing, in most cases, the separation of the second and third chromosomes. Cleavage could take place only when these chromosomes remained sufficiently close at the end of the maturation division to become incorporated into a single nucleus. Cleavage could include the other chromosomes only when these remained sufficiently close to the second and third to be included with them in a single nucleus. Further, cleavage failure in these cases is seen to be characterized by the failure of spindle formation in the female pronucleus, for at this stage no spindle is ever formed by a female pronucleus deficient in the second or third chromosomes.

This observation lends itself to orderly interpretation if we assume that an interaction between the second and third chromosomes, or portions of them, is necessary in order that a spindle be produced. Re-examination of the abnormal maturation figures shows the accordance of these with the assumption made. For here, at the first maturation division, the second and third chromosomes are often separated from each other, relatively few eggs developing normally at this time. These abnormal eggs, if the assumption is correct, should now fail to form a spindle for the second maturation division. This is found to be the case.

The assumption that no spindle is ever formed unless the second and third chromosomes are in close proximity leads to the corollary that the presence of a spindle at any time during development must have been preceded immediately by a close proximity of these chromosomes. How-

ever distorted the spindle may be, then, the preceding prophase must have been normal, containing in a single nucleus all the chromosomes to appear on the spindle at the succeeding metaphase. This test of the validity of the assumption made must be applied to the first maturation division, the last appearance of spindle formation in the eggs that are completely abnormal. This spindle, if the assumption is valid, must be preceded by a prophase in which all the chromosomes lie in a single nucleus, surrounded by a nuclear membrane. This is found to be true. No preparations have been found in which the nucleus during the prophase of the first maturation division was abnormal. In every case the germinal vesicle was single, containing within it all the chromosomes. Figure 10 shows the single germinal vesicle of an egg before laying. Figure 11 shows the nucleus of a later stage of development of an unlaid egg, in which the nuclear membrane has just broken down. Again the chromosomes are found in a single compact group. Figure 12 shows the anaphase of the first maturation division. The spindle is completely formed, and for the first time the chromosomes are seen to be widely separated from each other in the cytoplasm. In such an egg, where the second and third chromosomes no longer lie together, no orderly mitotic figures can again be formed and the egg can never develop into an adult. Thus at no stage of development have figures been found which will invalidate the assumption that the second and third chromosomes must be in close proximity for spindle formation to take place.

STURTEVANT'S hypothesis for the production of gynandromorphs and fourth chromosome mosaics suggests that a chromosome may be dropped from one pole of the cleavage figure. To determine whether this process ever occurs or not is difficult, since the formation of gynandromorphs is rare. The fact that no evidence of the elimination of chromosomes has been found cytologically is thus no proof that this process never occurs.

The cytologic preparations studied, however, indicate that gynandromorphs may be formed by a process simpler than that which STURTEVANT suggests; namely, by the addition of a chromosome to a deficient nucleus. This new hypothesis accounts as well as does STURTEVANT'S for the two types of gynandromorphs which he found. Figure 7 shows the possibility of formation of the first class of gynandromorph, that having exceptional male and normal female tissue. The figure shows a preparation in which first cleavage is in progress. The diploid set of chromosomes lacks a single X chromosome, which was excluded from the female pronucleus by the separation of the chromosomes at the first maturation division. Near the upper pole of the figure, beyond the centriole, a single X chromosome is seen, lying free in the cytoplasm. This chromosome must be one that had been eliminated during a previous division, since it does not lie in a posi-

tion indicating its elimination during the division in progress. Since this is the first cleavage the extra chromosome can only have been eliminated during the maturation divisions of the egg. It is therefore derived from one of the abnormal polar nuclei formed at that time. Further, this chromosome lies in what may become the path of the upper set of cleavage chromosomes when the division progresses. If, during this or later cleavages, the chromosomes at one pole of the cleavage figure lie close to the region occupied by the extra chromosome, the latter may become incorporated within that nearby cleavage nucleus. That one cleavage nucleus would then be enriched by this chromosome to contain the diploid set of X chromosomes, the remaining nuclei still being deficient for this chromosome and containing only the single X chromosome present before cleavage began. The deficient nucleus would thus lack a maternal X chromosome, missing since the formation of the female pronucleus. A gynandromorph would result in which the female parts, derived from the enriched nucleus are normal, and the male parts carrying a single X chromosome derived from the paternal set, are exceptional. This is the type of gynandromorph which was found by STURTEVANT to make up over 90 percent of those studied.

The egg pictured in figure 8 shows the possibility of formation of the second class of gynandromorphs. Again first cleavage is in progress but in this case the female pronucleus is complete. Above the complete maternal group of chromosomes is a vesicle containing an extra X chromosome which is not at this time entering into the cleavage. During cleavage the nuclear membrane surrounding this chromosome breaks down, setting the chromosome free in the cytoplasm as are those shown in figure 7. The extra chromosome may then pass to one pole of the cleavage figure, there to enrich one of the cleavage nuclei. If the egg pictured began its development as a normal male, the enriched nucleus now becomes XXY in chromosomal constitution, the other nucleus remaining XY as before the cleavage. A gynandromorph is thus produced in which the female tissues, derived from the enriched nucleus are exceptional female in constitution, and male tissues, derived from the unenriched XY nucleus are normal male. Genetically the result is the same as that obtained by STURTEVANT'S method, in which he suggests the formation of this type of gynandromorph by the elimination of an X chromosome from an egg which began its development as an exceptional female.

That chromosomes may be picked up during later cleavages is indicated by figure 9, in which, during the third cleavage, polar chromosomes are still seen to persist in the cytoplasm. Gradual degeneration of these chromosomes accounts for the fact that few gynandromorphs are found in which very small mosaic patches occur.

Such chromosome additions at later cleavages must be discussed more

fully than the simpler first cleavage additions which produce gynandromorphs that are half male and half female. The addition of chromosomes at later cleavages produces gynandromorphs that are more than half female or less than half female. Those that are more than half female are produced when a chromosome is added at a later cleavage to an egg that has already undergone addition at first cleavage. Those that are less than half female are produced by additions of chromosomes at later cleavages to eggs that have undergone no previous additions. STURTEVANT (1929) found that 45 percent of all the gynandromorphs are more than half female while only 15 percent are less than half female. To fit the addition scheme to these facts it is necessary to show that chromosome additions occur so frequently at first cleavage, and so frequently again at later cleavages, that 45 percent of the eggs are likely to suffer at least two successive additions. Estimates of the number of eggs affected by abnormality at each cleavage, if the elimination hypothesis is used as the basis for such estimates, do not lend themselves to the support of the addition scheme, as indicated by Dr. STURTEVANT in a personal communication to the writer. However, estimates based on the elimination hypothesis may not be applied directly to the addition scheme.

The error in such a direct application may be illustrated as follows. Cleavage abnormality, if that abnormality is elimination, produces male tissue. The extent of male tissue in the adult indicates, therefore, the developmental stage at which this specific abnormality occurred. Each gynandromorph which is one half or more than one half male has thus developed from an egg in which elimination occurred at first cleavage, or successively at first and later cleavages. This group of gynandromorphs includes 55 percent of the total, and indicates the frequency with which first cleavage elimination occurs. Addition of chromosomes, on the other hand, produces female tissue, and the extent of female tissue in the adult indicates the developmental stage at which this particular abnormality occurred. Here, each gynandromorph that is one half or more than one half female has developed from an egg in which addition of chromosomes occurred at first cleavage, or successively at first and at later cleavages. This group of gynandromorphs includes 85 percent of the total, and indicates the frequency with which first cleavage addition occurs. Such estimates can thus be made only when a specific mechanism is under consideration, and the estimates based on one mechanism do not apply when the other mechanism is considered.

Similarly, second cleavage elimination is characterized by gynandromorphs that are one quarter, three quarters or more than three quarters male. These total 35 percent. Second cleavage addition, however, is characterized by gynandromorphs that are one quarter, three quarters or more than three quarters female. These total approximately 53 percent.

Thus the elimination scheme implies that 55 percent of the eggs become abnormal at first cleavage and 35 percent at second cleavage. On this basis it is to be expected that 19 percent (55 percent × 35 percent) of the gynandromorphs will bear evidence of successive elimination of chromosomes at both first and second cleavage. STURTEVANT's counts of such gynandromorphs, characterized by the elimination scheme as being more than one half male, number 15 percent of the total. The addition scheme, however, leads to the expectation of 45 percent (85 percent × 53 percent) of gynandromorphs bearing evidence of successive addition of chromosomes at first and second cleavage. Counts of such gynandromorphs, characterized by the addition scheme as being more than one half female, number 45 percent of the total. The actual percentages here are not significant, because of the necessarily rough estimates of each class of gynandromorph made by STURTEVANT. They indicate, however, that either scheme may be used to explain the relatively large amount of female tissue found in the gynandromorphs.

The addition scheme may thus explain the formation of gynandromorphs and account for two peculiarities shown by them, namely, the absence of the maternal chromosome in over 90 percent of the gynandromorphs, and the fact that generally half or less than half of tissues of each of these gynandromorphs lack this chromosome. While the addition scheme presents no advantage over the elimination scheme in explaining the genetic data, it presents the advantage of a mechanical basis for the fact that it is always a maternal chromosome, derived from the egg, that is lacking in the deficient tissue. As the direct result of the abnormality of the first maturation division, this chromosome failed to be included in the female pronucleus and was thus lacking before cleavage began. When no additional chromosome is picked up by one of the cleavage nuclei the entire fly is deficient for this chromosome. When one of the nuclei produced by the first cleavage is enriched, only half of the tissue remains deficient for the maternal chromosome. A similar process during later cleavages will produce varying amounts of male and female tissue. The gradual degeneration of the extra polar chromosomes will reduce the frequency of this process in older eggs, making less likely the formation of gynandromorphs with small patches of mosaic tissue.

SUMMARY

1. Cytologic preparations of the eggs of the claret mutant type of *Drosophila simulans* are described.

2. The initial abnormality in these eggs is the separation of the chromosomes at each pole of the distorted spindle of the first maturation division.

3. The second abnormality is the failure of the second maturation

division in most eggs, and the reorganization of the chromosome groups at the end of this period, into an abnormally large number of nuclei, each containing an abnormally small number of chromosomes.

4. The third abnormality is the failure of cleavage in most eggs. This is correlated with the failure of a maternal spindle to appear in the absence of the second or third chromosomes from the female pronucleus.

5. These events are explained in the light of the abnormality of the first maturation division.

6. A new hypothesis is presented to account for the formation of gynandromorphs in this stock. The hypothesis is based on the cytologic data presented.

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EXPLANATION OF FIGURES

The figures were drawn with a Leitz 10× ocular and a Leitz oil immersion objective, N A 1.30, at table level with the aid of an Abbe camera lucida. A magnification of about 2540× was obtained. The figures have been reduced to one half in reproduction.

FIGURE 1.—Control stock. First maturation division. Five minutes after fertilization.

FIGURE 2.—Control stock. Second maturation division. The chromosomes at the extreme left will form the female pronucleus. Ten minutes after fertilization.

FIGURE 3.—Control stock. Reorganization of the four egg nuclei following the second maturation division. The nucleus at the left will form the female pronucleus. Eleven minutes after fertilization.

FIGURE 4.—Control stock. Three polar nuclei remaining at the periphery of the egg during cleavage. Twenty minutes after fertilization.

FIGURE 5.—Abnormal stock. First maturation division. The spindle is distorted and the chromosomes scattered at its poles. Five minutes after fertilization.

FIGURE 6.—Abnormal stock. Period of first cleavage. Twenty minutes after fertilization.

(a) Nine reorganized egg nuclei remaining at the periphery. Each contains a single chromosome. In the preparation these appeared in seven consecutive microscopic sections.

(b) Male pronucleus and three reorganized egg nuclei, that at the left containing the X chromosome, those at the right the second and third chromosomes. Figure reconstructed from two consecutive microscopic sections, one containing the male pronucleus and the autosome at the lower right, the other containing the X chromosome and the autosome at the upper right.

(c) Diagrammatic reconstruction of the above. The group of nuclei at the periphery at the left are the nine nuclei shown in (a), the central group the pronuclei shown in (b).

FIGURE 7.—Abnormal stock. First cleavage. Above the upper centriole is an extra X chromosome lying free in the cytoplasm. Twenty minutes after fertilization.

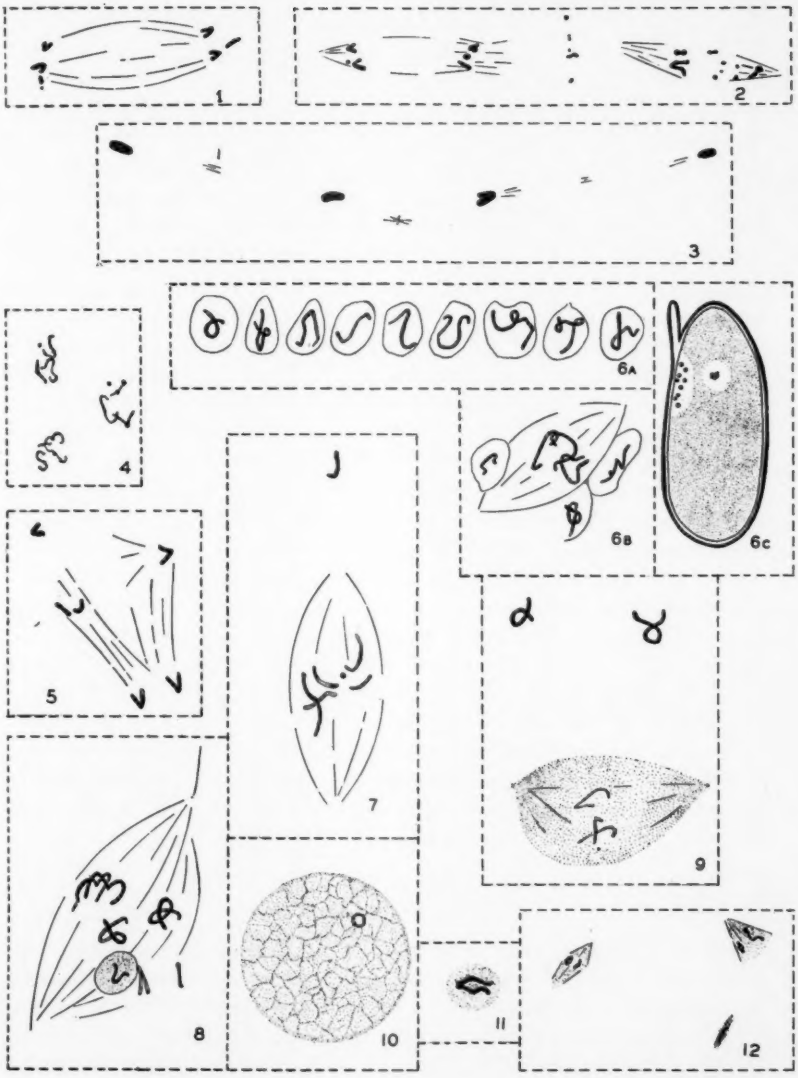
FIGURE 8.—Abnormal stock. First cleavage. The figure shows the paternal chromosomes at the left and the maternal at the right, these constituting a full haploid set. An extra vesicle containing a single chromosome lies above the maternal group. It has failed to form a spindle. Twenty-three minutes after fertilization.

FIGURE 9.—Abnormal stock. One of four nuclei of the prophase of the third cleavage. Two polar chromosomes are seen near it, lying free in the cytoplasm. Thirty-six minutes after fertilization.

FIGURE 10.—Abnormal stock. Prophase of the first maturation division. Single germinal vesicle. Unlaid, unfertilized egg.

FIGURE 11.—Abnormal stock. Shows a later stage than the previous figure. The nuclear membrane has broken down, the nucleus now lying at the dorsal surface of the egg. Unlaid, unfertilized egg.

FIGURE 12.—Abnormal stock. Shows a later stage than the preceding figure. The spindle is distorted, the chromosomes scattered into three widely separated groups. Unlaid, unfertilized egg.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GENETIC STUDIES ON THE NATURE OF HERMAPHRODITIC PLANTS IN ANTENNARIA DIOICA (L) GAERTN.

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IN MY previous papers on *Antennaria dioica* (1930, 1932, 1934) I dealt with the relations between color and sex, and considered the sex ratio as well as the inheritance of these characters. I made only passing reference to the fact that in addition to typical males and females, intermediate forms exist which may have more or less fertile organs of both sexes. The purpose of this paper is to give a genetic analysis of these aberrant forms.

The first classic paper on Antennaria by JUEL (1900) in which the author succeeded in demonstrating the parthogenetic reproduction of *Antennaria alpina*, contains the description of aberrant forms of *Antennaria alpina* and *A. dioica*, to which he refers in describing *A. dioica* as (1) intermediate hermaphrodites, (2) predominantly female hermaphrodites, and (3) aberrant females. Of these groups, the first resembles very much the typical staminate floret, yet is smaller in all dimensions and has a fertile ovary; the second group has two (instead of five) stamens, which, however, are not grown together to form a tube; the style is not as short and stiff as in the typical male but, on the other hand, does not show the elongated and parted appearance of the typical female; the third group is pure female, resembling only somewhat the staminate florets in the formation of the style and the external parts of the florets. For *A. alpina*, JUEL also describes an aberrant staminate type, which, however, has no pollen grains and no fertile germ cells. The same aberrant form seems to be more frequent than the normal one, for BERGMAN (1935) also recently reported it for *A. alpina*, and STEBBINS (1935) also describes it for the parthenogenetic species *A. fallax*, *A. parlinii* and *A. canadensis* in North America. It seems that in Antennaria aberrant forms are found wherever one makes a careful analysis.

DESCRIPTION OF THE NORMAL AND ABERRANT FLORETS OF ANTENNARIA DIOICA

Before reporting my own experiments with the various sexual forms of *A. dioica*, I shall give a more detailed description of them. The typical female floret of *A. dioica* has a slender pistil ending in two branches which bear stigmatic papillae. The corolla-tube encloses the style very tightly and there are no pollen sacs. The pappus-bristles are thin and their cells are almost confined to one row. The ovary is fertile. Text figure 1A shows the upper part of the corolla and the parted and protruding style. Figure 2

shows the apex of a pappus-bristle. Figure 3A represents a cross-section of the corolla and style.

The typical male floret has a thick button-shaped style which is very little divided or not divided at all. It shows no stigmatic papillae, yet on the external side it bears sweeping-hairs which sweep out the pollen grains when the latter are expelled from the sensitive pollen sacs on the external side of the style. Around the style stand five stamens grown together and forming a tube. This tube in its turn is surrounded by the corolla, which loosely incloses the pollen sacs. The pappus-bristles are broad and multicellular at the apex. Text figure 1H shows the upper part of the male floret. The appendices of the anthers protrude from the corolla. Figure 2G shows the upper part of the pappus-bristles. Figure 3C represents a section across the anthers and the corolla. (All corresponding figures of the different forms are drawn at the same magnification.)

The other pictures in figures 1 to 3 show all transitions between typical female and typical male florets. Figures 1B and 2B correspond to JUEL's aberrant female, a form that occurs very frequently, and that is represented here by plant number 250 (see also figure 3, plate 1). Its stigmata are somewhat stiffer and thicker and the corolla is a little wider; yet the floret is always purely pistillate, fertile, and never shows any evidence of anthers. During the blooming period it is easily distinguished from the typical female. Later, when the stigmata are dry, it may sometimes be mistaken for normal forms. (See plate 1, figures 6 and 7. Figure 6A represents the floret of a typical male, figure 6B of a typical female, and figure 7 of a floret of the same type as plant number 250.)

The predominantly female form as described by JUEL is seen in figures 1C and 1D and in 2C and 2D. It shows its less developed florets, resembling closely the type described above, yet it may contain quite well developed hermaphroditic florets. Figure 1D shows the button-shaped style with sweeping-hairs, while figure 1C in addition to the latter shows stigmatic papillae on the inside. The pappus-hairs (figures 2C and 2D) are somewhat broader than in pure females but much thinner than in pure staminate forms.

The next forms (plant no. 190, figure 1E and plant no. 253, figures 1F and 1G) approach the male type. The florets usually have male appearance although the style is often not enclosed in the anther tube but grows out from one side. The style frequently is bent as seen in figure 1G; self- or cross-fertilization thereby becomes difficult. The pappus-hairs are somewhat thicker than in the previous case. Nevertheless, these types are very similar to each other and can be distinguished only by their inheritance. The latter form probably ought to be put into one class with JUEL's "intermediate hermaphrodites." Figure 3B shows the parts of the floret

at the level of the anthers. There are only three anthers which are not joined in a tube; the style is situated on one side, and the corolla is as wide as in the males (plate 1, figure 8 shows two florets of plant 190 and plate 1, figure 5 shows one inflorescence of the same plant).

In addition to these differences, the male and female plants on the whole and the male and female heads in particular show distinct differences. This can be seen, without description, in figure 1, plate 1, where a male is shown at the left, a female at the right, and an aberrant female inflorescence in the center. The involucral bracts are much broader in the male, more elongated in the female. The fact that the color is different in each form and that there is a tendency in the males towards bright and in the females towards dark red colors has been referred to in one of my earlier papers (1930).

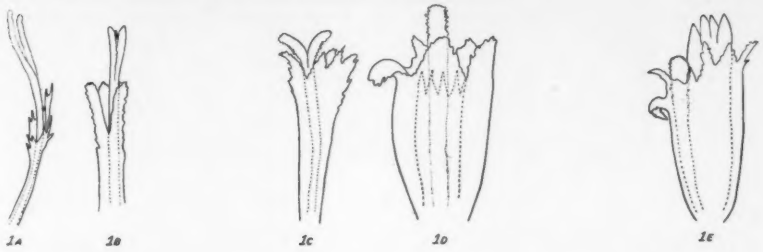
PROPORTIONS OF NUMBERS AND INHERITANCE OF THE DIFFERENT TYPES

In my previous papers I have attempted to analyze the numbers in which males and females appear. There are three possibilities: either as many males as females, or a preponderance of either males or females. I found that these proportions are due to a fertility factor which is transmitted together with the gene for sex and which allows a better growth inside the style to those pollen tubes which have at least one factor in common with the style tissue. The following possibilities have to be considered (table 1). The results of 42 crossing experiments are reported in tables 4–6 of my earlier paper (1934). I stated there that in certain crosses about half of the females belong to a more staminate type, but made no special report on them. In tables 2–5 of the present paper all these crosses are reprinted with special consideration of the aberrant forms. In addition, there are recorded 27 new crosses carried out during the last two years. Plants of the former crosses have bloomed for the first time since the last publication; hence, several figures relating to these had to be altered.

If we consider column 5, which contains the number of occurring forms, we find that in a series of cases there appear intersexes in addition to fe-

FIGURES 1–3

1A.—Floret of typical female, *FSFS*. 16×; 1B.—Floret of aberrant female, *F'SFS*, no. 250. 16×; 1C and 1D.—Florets of predominantly female hermaphrodite, *F'SF'S*, no. 306. 16×; 1E.—Floret of aberrant male hermaphrodite, *FSf'S*, no. 190. 16×; 1F and 1G.—Florets of aberrant male hermaphrodite *FS'f'S*, no. 253. 16×; 1H.—Floret of typical male, *FSfS;* 2A.—Hairbristle of typical female, *FSFS*. 50×; 2B.—Hairbristle of aberrant female *F'SFS*, no. 250. 50×; 2C and 2D.—Hairbristles of predominantly female hermaphrodite *F'SF'S*, no. 306. 50×; 2E.—Hairbristle of aberrant male hermaphrodite *FSf'S*, no. 190. 50×; 2F.—Hairbristle of aberrant male hermaphrodite *FS'fS*, no. 253. 50×; 2G.—Hairbristle of typical male, *FSfS;* 3A.—Cross-section of a female floret. 25×; 3B.—Cross-section of a hermaphrodite floret, no. 190. 25×; 3C.—Cross-section of a male floret. 25×.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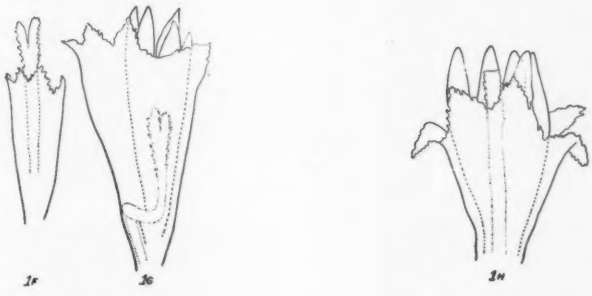
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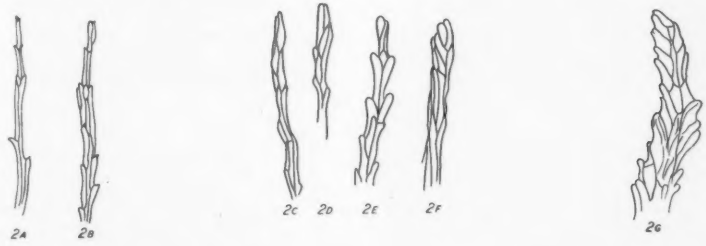
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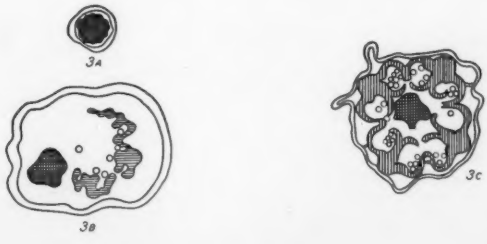
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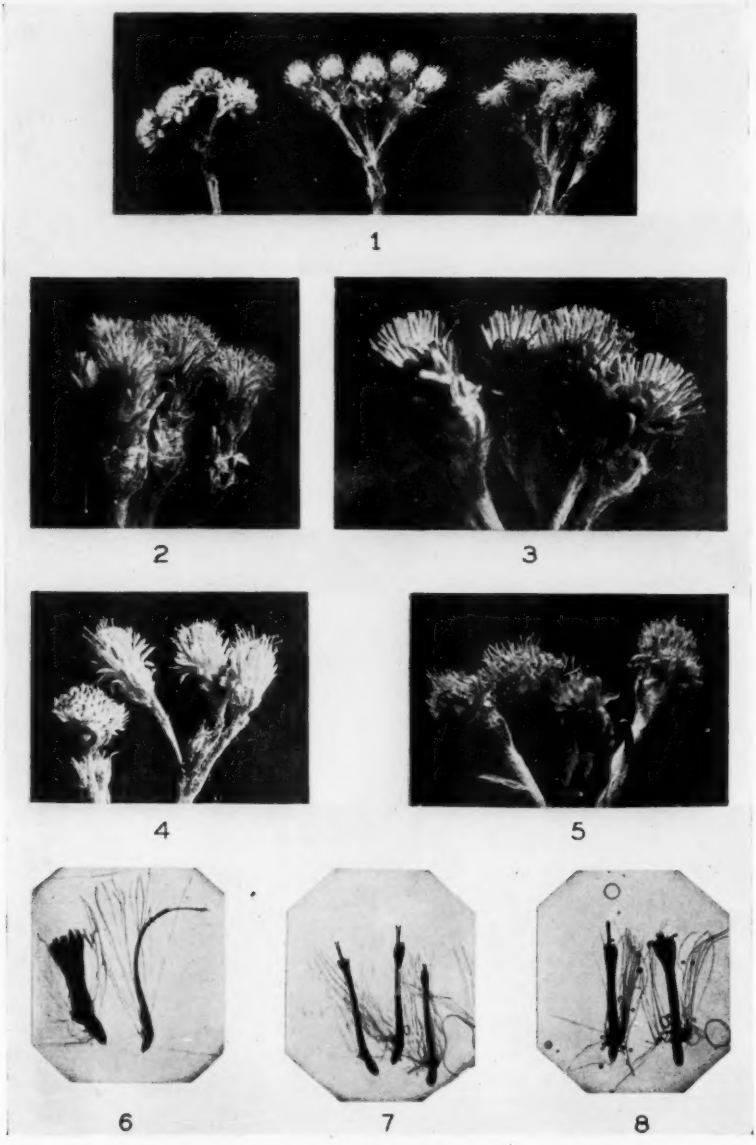

DESCRIPTION OF PLATE 1

FIGURE 1.—Male, hermaphrodite and female inflorescences of *Antennaria dioica*. Natural size.
FIGURE 2.—Inflorescence of a typical female. Genetic formula *FSFS*. Enlarged.
FIGURE 3.—Inflorescence of an aberrant female, no. 250. Genetic formula *F'SFS*.
FIGURE 4.—Inflorescence of a predominantly female hermaphrodite, no. 306. The head at the left resembles a male. Genetic formula *F'SF'S*.
FIGURE 5.—Inflorescence of an aberrant male hermaphrodite, no. 190. Genetic formula *FSf'S*.
FIGURE 6.—Micro-photograph of typical male floret (left) and typical female floret (right). 5×
FIGURE 7.—Micro-photograph of aberrant female floret, no. 255, *F'SFS*. 5×
FIGURE 8.—Micro-photograph of aberrant male hermaphrodite floret, no. 190, *FSf'S*. 5×

TABLE 1

PARENTS		PROGENY				CASE	
		FEMALES		MALES			
FEMALE	MALE	MANY	FEW	MANY	FEW		
FS FS	*FS fS*	*FS FS*		*FS fS*		1.	
FS'FS'	*FS fS*		*FS'FS*		*FS'fS*	2.	
FS'FS'	*FS'fS'*	*FS'FS'*		*FS'fS'*		3.	Same number of
FS FS	*FS'fS'*		*FS FS'*		*FS fS'*	4.	both sexes.
FS'FS	*FS fS*	*FS FS*	*FS'FS*	*FS fS*	*FS'fS*	5.	
FS'FS	*FS'fS'*	*FS'FS'*	*FS FS'*	*FS'fS'*	*FS fS'*	6.	
FS'FS	*FS'fS*	*FS'FS'*	*FS FS'*	*FS fS*	*FS'fS*	7.	
FS'FS	*FS fS'*	*FS FS*	*FS FS'*	*FS'fS'*	*FS fS'*	8.	
FS FS	*FS fS'*	*FS FS*			*FS fS'*	9.	Preponderance of
FS'FS'	*FS'fS*	*FS'FS'*			*FS'fS*	10.	females.
FS FS	*FS'fS*		*FS FS'*	*FS fS*		11.	Preponderance of
FS'FS'	*FS fS'*		*FS FS'*	*FS'fS'*		12.	males.

TABLE 2*

Equal number of males and females

1 NO.	2 PARENTS	3 ORIGIN	4 TOTAL NO.	5 ♀ : ⚥ : ♂	6 %♀	7 FORMULAE ♀	♂	8 TYPE OF FEMALE	9 TYPE OF INTERSEX	10 CASE
287	114×247	Sandh.×Krem.	138	69: 0: 69	50	*FSFS×FSfS*				I, 1
293	153×248	Kremn.×Krem.	212	106: 0:106	50					
515/16	453×442	265×319	209	98: 0:111	47					
512/22	446×442	319×319	85	42: 0: 43	49	*F'SFS×FSfS*		*F'SFS*		I, 2
319	240×308	Sandh.×Mosk.	120	36:26: 58	52	*F'SFS×F'SfS*		*F'SFS*	*F'SF'S*	I, 3
315	255×190	Kremn.×Krem.	89	19:23: 47	47	*F'SFS×FSf'S*			*F'SFS*	I, 4
423	351×307	273×Herlö	37	11: 8: 18	51	*F'SFS'×F'SfS*		*F'SFS'*	*F'SF'S*	V, 1
540	460×477	316×296	103	24:33: 46	55	*F'SFS'×FS'fS'*			*F'SFS'*	VI
222	191×164	Kremn.×Kremn.	18	11: 0: 7	61	*FSFS'×FS'fS*				VII, 1
302	241×164	Sandh.×Krem.	285	76:72:137	52	*F'SFS'×FS'fS*			*F'SFS'*	VII, 2
415	355×307	Herlö×Herlö	84	24:23: 37	56				*F'SFS'*	
304	254×162	Heidesh.×Kr.	74	38: 0: 36	51	*FS'FS×FSfS'*				VIII, 1
203	192×162	Kremn.×Kremn.	108	51: 0: 57	47	*F'S'FS×FSfS'*		*F'S'FS*		VIII, 2
204/20	192×172	Kremn.×Krem.	269	146: 0:123	54			*F'S'FS*		
281	59×242	Brünn×Sandh.	213	97:12:104	52			*F'S'FS*		

* The number appearing in the first column of the tables, for instance 287, refers to the number of the experiment. Experiments 200–222 were sown in 1928, 260–275 in 1929, 280–340 in 1930, 400–427 in 1932, and 500–558 in 1933. During the first year after the sowing only a few plants flower, the entire blooming period extending over many years. There is no reason to assume that wrong proportions of sexes were obtained during the first years (if we ignore the error due to smaller numbers), for as shown in a small table (1930) the first blooming of males and females occurs in the same proportions. In column 2 are recorded the numbers of the parents, as for instance 114×247, the female first. Column 3 gives the origin of the parents, either the place where they were collected (Sandhausen near Heidelberg; Heidesheim near Mayence; Kremnica and Brno in Czechoslovakia; Orellen in the Baltic; Moscow in Russia; Fellhorn in the Bavarian Allgaeu, and Herlö in Norway), or, in the case of plants resulting from a former cross, the number of this cross; thus, 269×319 indicates that the female which was used came from cross 269, the male from cross 319. Column 4 gives the total number of plants; column 5 the number of females, hermaphrodites and males; column 6 the number of females, the hermaphrodites being included among them; column 7 the genetic formula, and column 10 gives the key for each case with reference to table 1 of this paper. In columns 8 and 9 the type is recorded to which the females belong (if they are not *FF*) and the intersexes.

TABLE 3
Preponderance of females

1 NO.	2 PARENTS	3 ORIGIN	4 TOTAL NO.	5 ♀ : ⚥ : ♂	6 %♀	7 FORMULAE ♀ × ♂	8 TYPE OF FEMALE	9 TYPE OF INTERSEX	10 CASE
208	113×160	Sandh.×Krem.	62	44: 0: 18	71	*FSFS×FSfS'*			IX, 1
207	113×162	Sandh.×Krem.	75	52: 0: 23	69				
280	52×243	Brünn.×Sandh.	15	15: 0: 0	100				
282	66×177	Brünn×Sandh.	185	185: 0: 0	100				
300	239×177	Kremn.×Sand.	310	280: 0: 30	90				
295	165×175	Kremn.×Sand.	226	194: 0: 32	86				
206/91	130×160	Sandh.×Kr.	294	204: 0: 90	69				
289	114×162	Sandh.×Kr.	62	36: 0: 26	58				
211	131×162	Sandh.×Kr.	31	31: 0: 0	100				
538	458×476	Orellen×Hö	108	96: 0: 12	89				
272	255×175	Kremn.×Sdh.	85	61: 0: 24	72	*F'SFS×FSfS'*	*F'SFS*		IX, 2
268	251×242	Kremn.×Sdh.	101	63: 0: 38	62		*F'SFS*		
269	251×162	Kremn.×Krem.	58	56: 0: 2	97		*F'SFS*		
283	110×162	Sandh.×Kr.	200	198: 0: 2	99		*F'SFS*		
265	250×242	Kremn.×Sdh.	404	310: 0: 94	77		*F'SFS*		
213	110×160	Sandh.×Sandh.	14	13: 0: 1	98		*F'SFS*		
500/1	447×440	319×220	264	121: 86: 57	79			*F'SFS*	
502/3	448×440	319×220	467	209: 179: 79	83			*F'SFS*	
507	455×441	265×265	30	9: 10: 11	63			*F'SFS*	
523	481×441	283×265	17	8: 6: 3	83			*F'SFS*	
528/30	483×440	283×220	38	9: 24: 5	87			*F'SFS*	
413	306×257	Herlö×203	86	82: 0: 4	95	*F'SF'S×FSfS'*	*F'SFS*		IX, 3
284	110×196	Sandh.×Fell.	311	94: 168: 49	84	*F'SFS×F'SFfS'*	*F'SFS*	*F'SF'S*	IX, 4
294	161×196	Kremn.×Fell.	84	34: 50: 0	100		*F'SFS*	*F'SF'S*	
301	240×196	Sandh.×Fell.	386	170: 104: 112	71		*F'SFS*	*F'SF'S*	
401	349×156	253×Kremn.	29	12: 17: 0	100	*FS'F'S'×FS'fS*		*FS'F'S'*	X, 1
403	341×307	253×Herlö	6	4: 2: 0	100			*FS'F'S'*	
406	350×345	253×275	91	38: 33: 20	78			*FS'F'S'*	
407	350×307	253×Herlö	468	249: 125: 94	80			*FS'F'S'*	
408	350×156	253×Kremn.	593	178: 174: 241	60			*FS'F'S'*	
409	343×307	253×Herlö	22	9: 13: 0	100			*FS'F'S'*	

TABLE 4
Preponderance of males

1 NO.	2 PARENTS	3 ORIGIN	4 TOTAL NO.	5 ♀ : ⚥ : ♂	6 %♀	7 FORMULAE ♀	♂	8 TYPE OF FEMALE	9 TYPE OF INTERSEX	10 CASE
285	113×156	Sandh.×Krem.	100	44: 0: 56	44	*FSFS×FS'fS*				IX, 1
209/10	113×164	Sandh.×Krem.	165	63: 0:102	38					
202	130×156	Sandh.×Krem.	152	58: 0: 94	38					
201	130×164	Sandh.×Krem.	81	16: 0: 65	20					
286/88	114×106	Sandh.×Sand.	40	16: 0: 24	40					
217/90	114×164	Sandh.×Sand.	67	17: 0: 50	25					
508/9	445×156	300×Kremn.	19	6: 0: 13	31					
273	255×156	Kremn.×Krem.	39	14 25	36	*F'SFS×FS'fS*			*F'SFS'*	XI, 2
316	255×253	Kremn.×Krem.	108	26:21: 61	43	*F'SFS×FS'f'S*			*F'SFS'*	XI, 3
266	110×253	Sand.×Kremn.	48	20: 0: 28	42			*F'SFS'*		
314	306×253	Herlö×Kerm.	16	8 8	50	*F'SF'S×FS'f'S*				XI, 4
536	485×461	207×316	50	5: 0: 45	10	*FSFS×FS'f'S*				XI, 5
296	167×196	Kremn.×Fellh.	233	85 148	36	*FS'FS'×F'SfS'*				XII

males, usually in nearly the same number. If we add these to the females, a percentage of females is obtained (column 6) which corresponds to the figures of column VI in my earlier paper (1934). These intersexes are the aberrant forms described in this paper.

TABLE 5

Self fertilization

1	2	3	4	5	6	7	8	9	10
NO.	PARENTS	ORIGIN	TOTAL NO.	♀:⚥:♂	%♀	FORMULAE ♀ ♂	TYPE OF FEMALE	TYPE OF INTERSEX	CASE
551	469	315	40	12: 7:21	30	*FSf'S*×*FSf'S*		*FSf'S*	
544/45	463	315	44	11:11:22	25			*FSf'S*	
549	467	315	10	3: 2 5	30			*FSf'S*	
546	464	315	20	0:11: 9	0			*FSf'S*	
547	465	315	11	6: 2: 3	55			*FSf'S*	
550	468	315	9	3: 3: 3	33			*FSf'S*	
548	466	315	12	8: 1:13	67			*FSf'S*	
543	462	316	53	23:11:19	44			*FSf'S*	
542	461	316	12	4: 4: 4	33			*FSf'S*	
417	253	Kremn.	19	7: 7: 5	37			*FS'f'S*	

TABLE 6

Melandrium dioicum	*Antennaria dioica*
F'F':*MM* = 120:80 superfemales	*F F* :*MM* = 100:80 typical females
F F':*MM* = 110:80 superfemales	*F F'*:*MM* = 95:80 female intersexes
F F :*MM* = 100:80 typical females	*F'F'*:*MM* = 90:80 female intersexes
F'f' :*MM* = 84:80 male intersexes	*F f'* :*MM* = 73:80 male intersexes
F'f :*MM* = 80:80 male intersexes	*F f* :*MM* = 70:80 typical males
F f' :*MM* = 74:80 male intersexes	*F'f'* :*MM* = 68:80 inframales
F f :*MM* = 70:80 typical males	*F'f* :*MM* = 65:80 inframales
f' f' :*MM* = 48:80 inframales	*f' f'* :*MM* = 46:80 inframales
f' f :*MM* = 44:80 inframales	*f' f* :*MM* = 43:80 inframales
f f :*MM* = 40:80 inframales	*f f* :*MM* = 40:80 inframales

If we attempt now to give a genetic analysis of these forms, we may follow the explanation of G. and P. HERTWIG (1922) regarding the intersexes of Melandrium. This dioecious plant occasionally produces also hermaphroditic forms, but they are transformed males, as is shown by their anatomical structure. The HERTWIGS gave the following explanation of the forms. Since Melandrium is heterozygotic in the male, we must ascribe the formula *FFMM* to the female and *FfMM* to the male. The quantity of the female factor must hence be greater than that of the male factor, for instance, *F*:50; *M*:40; *f*:20. Thus in typical females, the female:male ratio *FFMM* = 100:80; in typical males, the female:male ratio *FfMM* = 70:80. There might be an increase of the quantities from

F to F' and from f to f' of 20 percent. In this case we have the following possibilities (table 6, left). Some of these aberrant forms could be identified by the HERTWIGS. The difficulty with their material as well as with mine lies in the wide range of variation. Inframales could not be obtained; they are probably inviable.

From the formulae for the intersexes it may be seen that they originate from the males since they all have f. This distinguishes them from the Antennaria intersexes.

HYPOTHESIS TO EXPLAIN THE ANTENNARIA INTERSEXES

The typical female is given the formula of $FFMM$ with $F = 50$, $M = 40$. The typical male is $FfMM$, f being 20 as in Melandrium. We assume a quantitative alteration from F to F', diminishing the quantity of F to $F' = 45$, while the quantity of f is raised to $f' = 23$. (There is no reason to raise or to diminish the quantities of F and f proportionately, as their alterations must have been brought about by independent mutation.) Thus we obtain the sexual forms of table 6, right. The aberrant female will then be $F'F'MM$ and the intermediate hermaphrodite $Ff'MM$. Only the latter is derived from the male form. In contrast to Melandrium here occur also $f'f'MM$ forms. All males resulting from self-fertilization must have this formula (table 5), while the HERTWIGS did not get plants, definitely known to be males, from self-pollination of hermaphrodites.

Considering tables 2–5 from the point of view of the genetic formulae, the fact must be stressed that the deviation from the chance sex ratio 1:1 is in no way altered by the modification of the quantity of the factors F and f into F' and f', if the aberrant forms are classified with the female and male forms respectively, according to whether they are $F'F$ and $F'F'$ or Ff'. The sex ratio depends on the fertility factor S or S' only (table 1). The proportions of typical to aberrant pistillate forms ought to be 1:1 whenever it appears. Any deviation must be ascribed to the range of variation which obscures the differences. It is always easier to state that there are two different types in the crosses than it is to classify the plants into these types. Thus in the crosses the FF' are considered typical female if together with them appear $F'F'$ females which were recorded as aberrant females. In other crosses $F'F$ was considered as aberrant form when no $F'F'$ appeared. Besides, the eye had to become accustomed to the subtle differences; in the first years (numbers below 304) $F'F$-flowers on account of these difficulties were considered as typical in most instances. Finally, the plants of the last year (1935) were sent to me dried, and in this condition small differences cannot be distinguished.

If in view of this, the assigned genetic formulae seem to be questionable, I should like to draw attention to some invariable features of the classification: 1. $F'F'MM$-forms were always considered as intersexes. 2. When

F'FMM and *F'F'MM* appeared together, the former were always considered as typical, the latter as intersexes. Crosses with the recently obtained male form *F'fMM* should be especially interesting. This form is considered an intersex by the HERTWIGS; in Antennaria it is an inframale. Crossed with normal females, however, intersexes must result, and a cross with the intermediate form *FF'MM* must give rise to hermaphrodites. The actual occurrence of these postulated types is demonstrated by one individual each in cases I,3, V,1, and XII, and by three individuals in case IX,4 (tables 2–4).

The correctness of our assumption seems assured by the great number of crosses with relatively few different plants, for which all crosses must show the same genetic formula. There are 69 crosses with 36 different plants: each plant appears in an average of four crosses, sometimes much oftener, and the genetic formula is seldom in doubt.

No information is available concerning the origin of the aberrant factors *F'* and *f'*. My first assumption that the factors would be somewhat different in the various countries, and that we are dealing with different geographical races in the sense of RICHARD GOLDSCHMIDT, has proved correct only in so far as the plants from Norway, Moscow and Allgaeu perhaps had a somewhat different quantity than the others, but the numbers are not sufficient to justify the assumption of factors *F''*, *f''*, etc. The plants from Central Europe were alike in that they showed both normal and aberrant forms.

A necessary complement of the analysis is the examination of the chromosomes, which I expect to finish during the next years as I possess fixed material from almost every plant. The chromosome number of *Antennaria dioica* has been determined by JUEL (1900) to be 12–14 haploid, the same result being obtained by BERGMAN (1935) who counted 28 chromosomes in the root-tips. My own rather superficial examinations show that in the embryo-sac and in the pollen mother cells chromosomes of very different forms and types appear which will perhaps make it possible to distinguish one of the chromosomes as the sex chromosome. In this case it remains to be determined whether and how the intersexes differ cytologically from the normal forms.

SUMMARY

In *Antennaria dioica* (L) Gaertn. a number of forms occur which according to their structure stand between the normal female and the normal male or surpass the latter. An explanation on a genetic basis is given for these forms and their inheritance investigated in a number of crosses.

I am very much indebted to Miss Lilli zur Nieden who enabled me to finish this investigation by carefully reaping and sending to me the plants which had flowered during 1935, and to Mrs. Anny Planet for her help with the English text.

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